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THE INHERITANCE OF SEX AT ENDOMIXIS IN PARAMECIUM AURELIA[10]

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Recently, Sonneborn[1] reported the discovery of a sexually differentiated race of *Paramecium aurelia*. In this race, *S*, every individual is of one of two sexes, called by him sex I and sex II; and the vegetative progeny of any individual are all of the same sex as the progenitor. When cultures of different sex are mixed, the individuals unite at once for conjugation; but when cultures of the same sex are mixed, no conjugation occurs. Sonneborn reported in detail the inheritance of sex at conjugation but gave only preliminary, qualitative results on its inheritance at endomixis. The present paper gives a precise and quantitative analysis of the segregation, inheritance and determination of sex at endomixis, and of certain related matters.

The inheritance of sex at endomixis was examined in three groups of individuals of the race *S* reported on by Sonneborn.[1] Each group consisted of a number of daily isolation lines descended vegetatively from a single individual. The three original individuals were closely related, but two of them were of sex I and one was of sex II. Samples from all the lines of the three groups were stained daily according to the method of Sonneborn[2] to determine when endomixis occurred. After each period of endomixis in each of the groups, all but one line was discarded; and a new set of lines was expanded from the one retained. To perpetuate the group a line was always selected of the same sex as the original progenitor. In this way, there were always maintained throughout the course of the work two groups of sex I and one group of sex II.

Segregation of Sex.—Sonneborn[1] found that some endomictic individuals give rise to two lines of descent differing in sex and that this segregation of sex occurs soon after the beginning of endomixis. The first question for analysis, therefore, was to ascertain the precise point at which this segregation occurs. As endomixis is a process which extends through several fissions, it was necessary to establish a definite point of reference for the study.

A brief description of the process will be necessary to make clear the subsequent account.

According to Woodruff and Erdmann,[3] endomixis begins with the gradual disintegration of the macronucleus. After the stage known as climax, there may or may not be a fission. In either case in each of the one or two individuals formed, two products of the micronuclei transform into new macronuclei through preliminary growth stages known as anlagen. These two anlagen are then segregated into separate individuals at the next fission, thus restoring the normal number of one macronucleus. In the subsequent fissions, the new macronuclei divide; and the fragments of the old macronucleus are irregularly segregated. Eventually the latter become resorbed, but they are visible in diminishing numbers for several fissions.

In order to ascertain the point at which sex is segregated, it was necessary to obtain and recognize those individuals in which had occurred the complete disintegration of the old macronucleus and the formation of the two anlagen of the new macronuclei. This was accomplished in the following way. Small mass cultures were set up consisting of the surplus animals from the isolation lines of the group to be tested. Endomixis was then induced by the method described by Sonneborn.[4] Observations were made at least every twelve hours to be sure that no conjugation had occurred. In no case was it ever found. At various intervals, a number of animals from the culture were stained with aceto-carmine to determine whether endomixis was occurring and if so, what stage the animals were in. When the culture was found to contain a large number of animals with two anlagen, some in earlier stages, some non-endomictics, and a very few or none with one anlage—i.e., after the first division after the formation of the anlagen—it was considered suitable for selecting animals in the two-anlagen stage. At this time, many of the animals with two anlagen are larger than most (or all) non-endomictics, and they may further be distinguished by their clear and narrow anterior end. Animals with these characteristics were chosen; and in every experiment some of those chosen, both before, during and after the selection of animals for the experiment, were stained in order to be sure that the selection was made accurately. That it was is shown by the fact that of 171 animals so chosen and stained 157 had two anlagen, 3 were in climax, 2 had only one anlage and 9 were non-endomictics. Those in the two-anlagen stage and probably those in climax were those required for the study. The non-endomictics were always eliminated by discarding those lines in which no fragments of the old macronucleus were found in one of the products of the second or third fissions. This method assures the selection of groups in which practically all the animals are in endomixis just prior to the first fission after the formation of the anlagen since, as the data show, not more than 3% of these groups were animals which were in endomixis prior to the formation of the anlagen or after the

first fission. This error is too small to appreciably affect the results.

The cultures to be tested for sex were obtained by allowing each of the selected endomictic individuals to multiply in from 4 to 5 drops of culture fluid until many animals were present and the food supply had begun to decline. Each culture was then tested for sex type by adding some of the individuals to each of two groups of animals: one group known to be sex I; the other, to be sex II. In nearly all cases conjugation occurred in one of these cultures only. The few exceptions to this are now under investigation. The sex of the animals tested was, of course, the opposite of that of the animals with which conjugation occurred.

Two extensive experiments were performed to determine the fission at which sex segregates at endomixis. In these experiments the two products of the first fissions of 181 individuals in the two-analgen stage of endomixis were isolated, and their progeny later tested for sex type. The two cultures from 96 of these individuals proved to be of the same sex, the two cultures from the remaining 85 individuals were of opposite sexes. Thus sex segregates at the first fission after the formation of the anlagen in approximately one-half the endomictic individuals.

In order to determine whether sex also segregates at a later fission, 353 products of the first fission after the formation of the anlagen were allowed to divide a second time; and, in each case, the two products of this second fission were isolated, and their progeny later tested for sex type. In 349 of the 353 cases, the two cultures thus produced proved to be of the same sex. In two of the other four cases, one of the cultures was of sex I; and the other formed pairs when mixed with animals of either sex. In the remaining two, one culture was of sex II; and the other reacted with both sexes. Three of the four exceptional cultures also contained conjugants at the time they were tested for sex type. None of these four cultures was tested for sex type sooner than six days after endomixis. Whether these four cultures actually represent exceptions to the general rule that the segregation of sex occurs only at the first fission after the formation of the anlagen or whether they can be explained on some other basis remains for a more thorough investigation to answer. It may therefore be concluded that sex never, or very rarely, segregates at a later fission than the first after the formation of the anlagen.

Sex Ratios.—The two sexes were produced in three different ratios in the six experiments that were performed. In four of the experiments the following numbers were obtained:

EXPERIMENT	1	2	3	4	TOTAL
Sex I	71	26	66	88	251
Sex II	63	21	55	94	233

These results are fairly close to the exact 1:1 ratio of:

EXPERIMENT	1	2	3	4	TOTAL
Sex I	67	$23^1/_2$	$60^1/_2$	91	242
Sex II	67	$23^1/_2$	$60^1/_2$	91	242

In another experiment 128 individuals of sex I and 63 of sex II were obtained, this being almost exactly a 2:1 ratio (mathematically, $127^1/_3 : 63^2/_3$). In a third experiment 63 individuals of sex I and 137 of sex II were obtained, which is close to a 1:2 ratio (mathematically, $66^2/_3 : 133^1/_3$).

Sonneborn found three different classes of endomictics: those that give rise solely to sex I; those that give rise to both sex I and sex II; and those that give rise solely to sex II. The same three classes were found in the present work. If random distribution of the two sexes to the two products of the first fission occurs, the proportion of each of these classes produced depends on the ratio in which the two sexes occur. With a 1:1 sex ratio, a 1:2:1 distribution of the three classes is expected; with a 2:1 sex ratio, a 4:4:1 distribution; and with a 1:2 sex ratio, a 1:4:4 distribution. In the four experiments which gave a 1:1 sex ratio, the following numbers of endomictics of the three classes were obtained:

EXPERIMENT	1	2	3	4	TOTAL
All sex I	19	5	14	21	59
Sex I and sex II	31	11	31	45	118
All sex II	13	3	10	22	48

With an exact 1:2:1 ratio, the following would be the numbers:

EXPERIMENT	1	2	3	4	TOTAL
All sex I	$15^3/_4$	$4^3/_4$	$13^3/_4$	22	$56^1/_4$
Sex I and sex II	$31^1/_2$	$9^1/_2$	$27^1/_2$	44	$112^1/_2$
All sex II	$15^3/_4$	$4^3/_4$	$13^3/_4$	22	$56^1/_4$

In the experiments which gave a 2:1 sex ratio, the numbers of endomictics of each of the three classes were 42:40:11, almost identical with the theoretical numbers ($41^1/_3 : 41^1/_3 : 10^1/_3$). In a third experiment, in which a 1:2 sex ratio was obtained, the number of endomictics of each of the three classes were 13:36:46 which is fairly close to the theoretical 1:4:4 ratio of $10^5/_9 : 42^2/_9 : 42^2/_9$. Therefore it can be concluded that the distribution of the two sexes to the products of the first fission after the formation of the anlagen is purely random.

Change of the Sex Ratio in a Single Line of Descent.—In two of the three groups examined, a different sex ratio was obtained in the second test from that obtained in the first. In one, the ratio changed from 1:1 to 2:1; in the other from 1:2 to 1:1. The two groups had a similar history between the tests. Each went through many vegetative fissions; each went through endomixis, the first once, the second twice; but neither went through con-

jugation. Therefore the change of ratio must have occurred either at vegetative fission or at endomixis, though neither of these processes necessarily involves a change of ratio since the third group went through both without changing its ratio. However, change of ratio must occur with considerable frequency since it was found in two out of the three groups twice tested.

Sex Determination.—The relations reported here for endomixis and those reported by Sonneborn[1] for conjugation are very similar. In both processes sex is segregated to different individuals at the first fission after the formation of the anlagen; and, at this same fission in both, the two new macronuclei that are formed are also segregated. Thus it is extremely probable that sex is determined by the macronucleus and that two different types of macronuclei can be formed: one determining sex I; and one determining sex II. Thus far sex determination at endomixis is just like that reported by Sonneborn for conjugation in this race. However, the experiments reported here show that after endomixis there are at least three sex ratios while Sonneborn reports only one, the 1:1 ratio after conjugation.

Sonneborn has suggested two possible chromosome mechanisms to account for the facts that he observed. These apply equally well to endomixis when a 1:1 ratio occurs. However, modifications are needed to explain the 2:1 and 1:2 ratios and also to explain the change of ratio. What the chromosome mechanism actually is can only be determined after a more thorough study.

Relation of the Results to Other Problems.—The results reported here have a bearing on several other problems of protozoölogy.

(1) It has been held in some quarters that endomixis is a variable reaction to diverse environmental factors and not a definite process as is conjugation. The preciseness of the ratios and of the segregation of sex as shown by these experiments indicates, on the other hand, that endomixis is quite as regular and exact a process as is conjugation.

(2) Diller[5] contends that much of what has been called endomixis is in reality autogamy or self-fertilization. While perhaps the close similarity of sex inheritance at conjugation and at endomixis seems to indicate that this is the case, actually the present results neither confirm nor contradict this contention but could equally well be explained on the basis of endomixis as described by Woodruff and Erdmann[3] or on the basis of autogamy as described by Diller.[5]

(3) The origin at endomixis of genetic diversities in other characters than sex has been reported by Erdmann,[6] Parker,[7] Caldwell[8] and Sonneborn and Lynch.[9] The fact that within a clone the macronuclei of different products of the first division after the formation of the anlagen at endomixis may differ in their sexual potency makes it seem possible that they may also differ in their potency in relation to other characters, thus explaining to a

degree the origin of hereditary diversities at endomixis. To what extent, if at all, this may be true remains for future investigation to discover.

[1] Sonneborn, T. M., *Proc. Nat. Acad. Sci.*, **23**, 378–385 (1937).
[2] Sonneborn, T. M., *Genetics*, **21**, 503–514 (1936).
[3] Woodruff, L. L., and Erdmann, R., *Jour. Exptl. Zoöl.*, **17**, 425–518 (1914).
[4] Sonneborn, T. M., *Biol. Bull.*, **72**, 196–202 (1937).
[5] Diller, W. F., *Jour. Morph.*, **59**, 11–67 (1936).
[6] Erdmann, R., *Arch. f. Entwmech.*, **46**, 85–148 (1920).
[7] Parker, R. C., *Jour. Exptl. Zoöl.*, **49**, 401–439 (1927).
[8] Caldwell, L., *Jour. Exptl. Zoöl.*, **66**, 371–407 (1933).
[9] Sonneborn, T. M., and Lynch, R. S., *Genetics*, **22**, 284–296 (1937).
[10] The author wishes to acknowledge his great indebtedness to Dr. T. M. Sonneborn for his constant interest and many valuable suggestions throughout the course of the work.

A THIRD LETHAL IN THE T (BRACHY) SERIES IN THE HOUSE MOUSE

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Congenital absence of the tail in a strain of mice first isolated by Dobrovolskaia-Zawadskaia and Kobozieff (1932) has been shown (Chesley and Dunn, 1936) to be due to interaction between the dominant gene for Brachyury *T* (Dobrovolskaia-Zawadskaia, *et al.*, 1934; Chesley 1935) and a recessive allele t^0. This strain, known as Line A, breeds true to taillessness because of a balanced lethal condition. The genetic composition of tailless animals of this line is Tt^0; the homozygotes *TT* die at 10–11 days after fertilization; t^0t^0 embryos die shortly after implantation (6th–7th day) and only the compounds Tt^0 survive. *T* and t^0 show no crossing-over and are probably alleles.

In a second tailless line,[1] also isolated by Dobrovolskaia-Zawadskaia and Kobozieff (1932) and designated as Line 29, a similar situation has now been found. Data in support of this statement are given in table 1. The strain breeds true to taillessness and produces small litters (Exp. 1). Crosses of tailless animals from this line by normal-tailed animals produced normal and short-tailed (Brachy) progeny (Exp. 2), indicating that Line-29 tailless is heterozygous for Brachyury (*T*). As in crosses of Line-A tailless, the ratios from Line-29 tailless females approximate 1 normal: 1 Brachy; those from tailless males indicate a marked excess of normals (Exp. 3). All F_1 normals when tested by Brachy ($T+$) transmitted taillessness,

indicating that all had received a recessive gene t^1 from the tailless parent. Females heterozygous for this recessive, when crossed to Brachy, produced clear ratios of 1 normal: 1 Brachy: 1 tailless (Exp. 4); similar normal males produced the same three classes of offspring but always showed a marked deficiency of Brachys (Exp. 5). The reasons for this sexual difference, which is even greater than in hybrids from Line A, are being studied further.

Matings between such F_1 normals produced only normal progeny at birth (Exp. 6), but examinations of the uteri of such females show that

TABLE 1

RESULTS OF MATINGS OF TAILLESS MICE OF LINE 29 AND OF TESTS OF THEIR DESCENDANTS

EXP. NO.	MOTHER	FATHER	OFFSPRING NORMAL	BRACHY	TAILLESS	TOTAL	LITTER SIZE
1	L-29 tailless	L-29 tailless			244	244‡	4.07
2	L-29 tailless	Normal*	32	26		58	5.8
3	Normal*	L-29 tailless	135	17		152	6.2
4	F_1 normal (from 2 & 3)†	Brachy	440	219	226	885	7.5
5	Brachy	F_1 normal†	228	31	153	412	7.7
6	F_1 normal	F_1 normal	78			78	5.2
7	F_1 Brachy	F_1 Brachy	18	36		54	5.4

* Inbred Bagg albino.

† Includes other known heterozygotes like $F_1(t^1+)$.

‡ Of these, 14 had small bony non-flexible stumps at the end of the pelvis. These breed like typical tailless animals and are probably identical with other Line-29 tailless animals as far as T and t^1 are concerned.

one class of embryos has died shortly after implantation. These are presumably the t^1t^1 homozygotes (Chesley and Dunn, 1936). The F_1 Brachy animals do not transmit t^1 but behave as ordinary Brachy animals from stock (Exp. 7).

These results, and others to be reported in detail later, are all consonant with the interpretations already applied to Line-A tailless; that is, tailless animals of Line 29 are Tt^1; T and t^1 show no crossing-over and are probably alleles; both homozygous combinations TT and t^1t^1 are lethal, but the compound of two lethals Tt^1 is viable.

Although *inter-se* matings of tailless animals within Line A or within Line 29 produce only tailless offspring, crosses of Line-A tailless by Line-29 tailless produce both tailless and normal-tailed offspring (Kobozieff 1935; Chesley and Dunn 1936). We assumed that the recessive alleles

in these two lines were different and that the cross might be represented as follows:

	Line-A tailless		×	Line-29 tailless	
P_1	Tt^0			Tt^1	
F_1	TT	Tt^1		Tt^0	t^0t^1
	dies	tailless		tailless	normal

This assumption has now been tested and confirmed. The pertinent data are given in table 2.

TABLE 2

				TAILLESS	NORMAL
Exp. 1	L-29 tailless (Tt^1)	×	Line-A tailless (Tt^0)	81	41
Exp. 2	F_1 normal t^1t^0	×	Brachy T +	78	77

The first experiment shows that the 2:1 ratio predicted from the above assumption is realized. This ratio would be expected if T and t^1 or T and t^0 segregate in the sperm in 1:1 ratios. It was necessary to assume abnormal ratios for this segregation in order to explain results from Tt^0, Tt^1, $+t^0$ and $+t^1$ males. The reasons for this discrepancy are now being sought. Kobozieff (1935) obtained from the cross of Line 29 by Line A: 14 tailless and 15 normal; the numbers are probably too small to constitute a serious disagreement with the hypothesis advanced here.

The second experiment shows that *all gametes* of the F_1 normals transmit a recessive allele which, when combined with T, produces taillessness. Crossing-over between t^0 and t^1 would result in gametes transmitting neither t^0 nor t^1 and, when combined with T, this would produce the Brachy condition. No Brachys have been found among 155 progeny; consequently crossing-over between t^1 and t^0 has not occurred, and we may thus regard these two genes as alleles. It is again noteworthy that the compound of two lethal alleles (t^0t^1) is viable. The results in Exp. 2, table 2, were obtained only from t^0t^1 females. Seven t^0t^1 males were tested with fertile females for from 5 to 7 months, but all proved to be infertile. Although this compound is viable, it appears to produce sterility in the males.

The evidence of allelism of t^0 and t^1 brings the number of probable alleles at this locus to at least four. In addition to T, t^0 and t^1, Reed (1937) has shown that the dominant tail mutation Fused (T^f) is closely linked with T and is probably an allele. Another tailless line from Dr. Dobrovolskaia-Zawadskaia (Line 19) contains a recessive allele of T which behaves like t^0 and t^1 but which is different from t^0 and possibly from t^1. Evidence on this point will be published later.

It is evident that several mutations affecting the structure of the tail have occurred at or near one locus. Since three of these (TT, t^0t^0 and t^1t^1) have early lethal effects when homozygous, while one (T^yT^y) appears to be less viable than normal (we have noted an embryonic anemia in this form), it is noteworthy that all of the compounds so far observed (TT^y, T^yt^0, Tt^0, Tt^1, t^0t^1) are more viable than either parental homozygote. In fact the compound of two recessive lethals t^0t^1 shows no phenotypic departure from wild type. Since the evidence of allelism of these two genes is now strong, it can be concluded that these mutations, and probably T and T^y as well, affect different primary processes which are essential to survival; that is, the process which is rendered abnormal by t^0t^0 is not the same as the process affected by t^1t^1. The combinations $T+$, TT^y, Tt^0, Tt^1, T^yT^y, T^y+, T^yt^0 and T^yt^1 apparently all affect the early organization of the axial structures, resulting in abnormalities of the tail or in its absence, so that the effects of these genes converge on morphogenetic processes fundamental to organization of these regions, while they retain a degree of independence with respect to certain other vital processes. The degree and nature of this differentiation at one genetic locus is being studied by embryological methods.

[1] We are greatly obliged to Dr. Dobrovolskaia-Zawadskaia for placing this material at our disposal. Line 29 originated in her laboratory from outcrosses of a tailless male from the original Brachy stock to two females of an unrelated normal stock followed by inbreeding among the tailless descendants of these crosses. The tailless condition in this stock bred true from its origin (Kobozieff 1935, p. 296). In our laboratory it was necessary to outcross this line to an inbred normal stock (Bagg albino) and to recover it by inbreeding tailless descendants.

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AN ACCELERATING EFFECT OF NORMAL "RING-GLANDS" ON PUPARIUM-FORMATION IN LETHAL LARVAE OF DROSOPHILA MELANOGASTER

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In several groups of insects it has recently been shown that hormones are concerned in moulting, pupation and metamorphosis (for literature, see Bodenstein 1936, Wigglesworth 1937). In connection with these problems the mutation "lethal-giant" in *Drosophila melanogaster* is of a special interest, since here there is a cessation of development at the beginning of pupation (Hadorn 1937). This paper deals with the question of whether it is possible to influence the process of puparium-formation of lethal larvae by injecting into them different tissues from genetically normal individuals. Since the study of this problem has led to the finding of a special "pupation-gland" the experiments may contribute not only to the physiology of lethals but also to the general physiology of insects.

1. *Material.*—The gene lethal-giant (*lgl*-Chrom. 2, 8±, found and located by Doctor C. B. Bridges, to whom I am grateful for unpublished information and for stocks) is kept in the balanced stock *lgl cn bw sp²/Cy*. In such a balanced stock three types of fertilized eggs are formed: (1) *Cy/Cy*, (2) *lgl cn bw sp²/Cy* and (3) *lgl cn bw sp²/lgl cn bw sp²*. Individuals, homozygous for the Curly-chromosome (*Cy*) die either in the egg or as small larvae. Individuals homozygous for *lgl* grow through the three larval instars. The combination of cinnabar (*cn*) and brown (*bw*) in homozygous condition is characterized by almost colorless Malpighian tubes (Beadle 1937). On the basis of this character the homozygous *lgl* larvae of the third instar can be distinguished and separated from the non-lethal heterozygotes of the same culture (*lgl cn bw sp²/Cy*), which have yellow Malpighian tubes. Eggs were collected at two-hour intervals, so that the age of the larvae of any experimental series was known within this limit.

2. *Puparium-Formation in Untreated lgl Larvae.*—When a larva of normal genetic constitution is ready for pupation, it everts its anterior spiracles and ceases to move. The cuticle then becomes hardened and darkened and develops the characteristic pupal shape. Within such a puparium the real pupa develops by evagination of the imaginal discs.

In larvae homozygous for *lgl*, puparium-formation may occur in a morphologically normal manner. But since the imaginal discs have degenerated at this time, no pupae can be formed within the puparia. We may call such a formation a "pseudopupa" (Hadorn 1937). With respect to pu-

parium-formation, lethal and normal larvae differ considerably in the time at which this process takes place. Normal larvae, in this case also the heterozygotes, pupate during the 5th day after the egg is laid (25°C.). The *lgl* larvae never start to form puparia before the beginning of the 7th day and there is a great variability among individuals of the same age. The second curve in figure 1 shows a percentage-distribution of a group of 220 individuals. Only 50% of them have formed puparia at $8^1/_2$ days. On the 11th day 10% are still in the larval stage. Some of these form puparia later, while others never do so at all. In such cases the larval life may be extremely long (up to 25 days). The shape of the puparium is not always

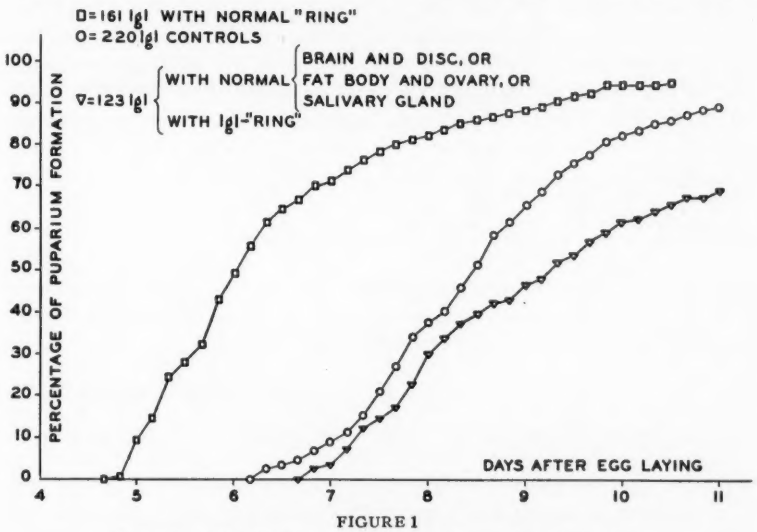

FIGURE 1

Percentage distribution showing time of puparium-formation.

normal. There may be incomplete eversion of the spiracles, or flattening of the dorsal head skin may not occur.

3. *Influence of an Implanted Genetically Normal "Ring-Gland."*—Though puparium-formation of Calliphora has been shown to be dependent on a hormone secreted somewhere in the head (Fraenkel (1935)), in the present case the question arises as to whether the delay in puparium-formation observed in the lethals is due solely to a lack of the necessary hormone or to a more general retardation involving both the acting and the reacting organs. An attempt was therefore made to find an organ of normal constitution which could act as a source of a pupation hormone. Of the different tissues tested by transplantation experiments none but a small body which is located dorsally between the two hemispheres of the "brain"

(supra-oesophageal portion of the central nervous system) had an accelerating effect of the formation of puparia in lethals.

This organ was described first by Weismann (1864) in his classical work on the postembryonic development of the Muscidae; he called it the "Ring" and suggested that it might function as a supporting structure for the "dorsal vessel" which goes through the ring. Many later investigators do not mention the ring at all. Lowne (1890–1895) states that in Calliphora the ring grows rapidly in "resting larvae" preparing for pupation.

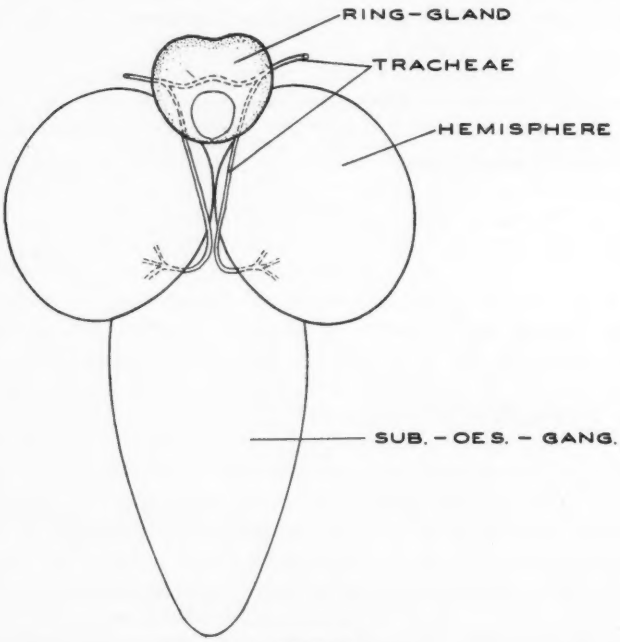

FIGURE 2

Location of the ring-gland of a Drosophila larva in respect to the central nervous system. (Semi-schematic.)

In *Drosophila melanogaster* I have found this ring to be similar in shape to that reported by Weismann for *Calliphora vomitoria*. Figure 2 shows its typical form and location with respect to the larval central nervous system. A detailed description of its structure and development cannot be given here. But it may be stated that the ring has a characteristic histology which makes it easy to identify in sections. The cells and nuclei are large and the cytoplasm deeply staining. The pattern formed by the tracheae which penetrate the ring is so characteristic that the latter may be located

without difficulty. It is easily separated from other tissues by means of dissecting needles.

Since it has been shown that the ring has a glandular function, the term "ring-gland" may be used so long as no homology to other structures in other insects has been established.

Experiments.—Using the method of Ephrussi and Beadle (1936) ring-glands from normal larvae were transplanted to a total of 161 *lgl* larvae. Hosts and donors were taken from the same 2-hour group; the donors were almost ready for puparium-formation. The glands were injected into the posterior portion of the larvae. Cultures were kept at 25°C. The experiments were run in rather small series. The time after operation at which puparium-formation for the different individuals occurred was noted and this was compared with the time of puparium-formation in non-injected control series of *lgl* larvae from the same 2-hour group (table 1, *a* and *b*). Observations were made every four hours during the day. Table 1 shows

TABLE 1

	NUMBER OF INDIVIDUALS	NUMBER OF SERIES	INTERVAL IN HOURS BETWEEN TIME OF IMPLANTATION AND PUPARIUM-FORMATION: FORMATION OF FIRST PUPARIUM — RANGE	FORMATION OF FIRST PUPARIUM — MEAN AND S. E.	FORMATION OF PUPARIA IN 50% OF INDIVIDUALS — RANGE	FORMATION OF PUPARIA IN 50% OF INDIVIDUALS — MEAN AND S. E.
(*a*) *lgl* with implanted normal ring-gland	161	14	15–23	18.6 ± 0.21	20–78	30.1 ± 1.20
(*b*) *lgl* controls without implant	220	12	30–79	49.8 ± 0.64	70–100	86.6 ± 0.58
(*c*) *lgl* with various implants, exclusive of normal ring-gland	123	13	41–80	65.4 ± 1.03	78–144	97.7 ± 2.27

first the data for the time when in the different series the first puparium was observed. The time given for the untreated control series (*b*) is measured from the time when the experimental series (*a*) were injected. The difference between the two means is 46.4 times its standard error and there is no overlapping even for the maximal range. The table shows further the figures for the time interval which elapsed before puparium-formation took place in 50% of the individuals in each series. Here again the difference between the two means is statistically significant (36.5 times its standard error). Curves 1 and 2 in figure 1 demonstrate percentage-distributions of all series. It can be seen that 50% of the injected lethals formed puparia before any of the 220 controls started. From the later course of the curves it can be seen that 75% of the injected larvae had formed puparia at $7^1/_4$ days after egg-laying, while the untreated controls required $9^1/_2$ days to reach the same percentage of puparia. For the last 25%, the difference between the two groups is smaller. Although puparium-formation took place in certain series in 100% of the cases, however, for the injected

group as a whole at the 11th day 5% of larvae were still present. Some of these formed a puparium later; others died as larvae in the manner described for the non-injected lethals. As there is always a possibility that the operation may not be successful, we can assume that some or possibly all of the individuals which formed puparia later or not at all, did not contain an implant.

4. *Implantation of Other Tissues.*—Since in Lepidoptera the "brain" is supposed to be the source of a pupation hormone (Kopeć 1922; Kühn and Piepho 1936), it is important to test its effect on *lgl* larvae. In 36 such individuals a *"brain"* (hemispheres) *without the ring-gland* was injected with the result that no accelerating influence whatsoever was observed. The injected larvae formed puparia even later than the average of the non-injected *lgl* controls. Together with the "brain" was injected either the eye-antennae-disc or the sub-oesophageal part of the central nervous system, so that we also know that these structures have no influence.

In a second group of 42 individuals the implant consisted of a large portion of the *fat body containing an ovary.* Here again no acceleration of puparium-formation was observed. The same lack of effect was found for *salivary glands* (22 cases).

The data reported above show that the acceleration of puparium-formation is not a general and non-specific effect of any tissue of normal constitution, but is due to a specific activity of the ring-gland. The question now arises as to whether the presence of a supernumerary ring-gland, regardless of its genetic constitution, causes the effect. This question was answered by injecting *ring-glands from lethal* donor larvae into lethal hosts. Since in a total of 23 individuals no acceleration was observed we conclude that the host and implanted *lgl* ring-glands together either do not furnish a sufficient amount of hormone or do not produce any at all during the time when the normal gland is active. In table 1 and curve 3 of figure 1, all the different injected series which did not contain a genetically normal ring-gland are grouped together. A comparison with the data and curves of the two other groups shows that, where anything other than a normal ring-gland is injected, puparium-formation occurs even later than in the untreated controls. This is true for most of the single series as well as for the group as a whole. It is obvious that, whatever the influence of the operation as such may be, it would not accelerate puparium-formation but rather retard it. Therefore, it is concluded that the accelerating effect obtained with normal ring-glands is entirely the result of an activity of the injected cellular material.

5. *Discussion.*—The experiments show that the retardation of the process of puparium-formation characteristic of the homozygous *lgl* larvae is not due to an inability of the reacting material (larval skin) to undergo

changes typical for pupation. The puparium can be formed in a lethal larva after a genetically normal ring-gland has been present for 15–23 hours. The retardation in the untreated lethals therefore seems to be the consequence of the absence of some substance normally secreted by the ring-gland.

A detailed histological study is needed to determine whether there are temporary or permanent differences between ring-glands of normal and lethal larvae. At present we can say that there is no indication of cellular degeneration in a *lgl* ring-gland at the end of the 5th day, while at this time the imaginal discs of *lgl* larvae have completely degenerated.

Since most of the untreated lethal larvae are able to form puparia, although this process is greatly retarded, the difference between the action of the *lgl* gene and its normal allele, so far as the process of puparium-formation is concerned, is perhaps quantitative. This difference may be of such a nature that the amount of hormone which is necessary for puparium-formation reaches the required threshold at different times in the two classes of larvae. However, we do not know whether the ring-gland of the lethals begins to secrete later in development than does a normal ring-gland or whether it becomes functional at the same time but produces the substance in a smaller amount. In either case the threshold would be reached later than in normal larvae. On the basis of such a quantitative interpretation an implanted normal ring-gland would supply to the lethal host enough hormone to bring it to threshold concentration earlier than in the untreated lethals.

Since the imaginal discs of *lgl* larvae were quite degenerate at the time normal ring-glands were implanted, no development beyond the puparium-formation was possible; we therefore do not yet know whether the ring-gland normally has any other activity besides its influence on puparium-formation.

Fraenkel (1935) showed by means of constriction experiments that only those parts of a Calliphora larva which contained the "brain" region pupate when the operation is made during a certain larval stage. Bodenstein (1936) briefly reported similar results obtained on Drosophila. Fraenkel concluded that either the "ganglion" (central nervous system) or something in its immediate neighborhood furnished the pupation hormone. Since the experiments reported in the present paper prove that for Drosophila the ring-gland and no other parts of the brain region can accelerate puparium-formation, it is probable that the ring-gland is also the source of the pupation hormone in Fraenkel's experiments.

Although Fraenkel claims "there exists no organ in the fly larva which can be homologized with the corpora allata in other insects," it should be emphasized that the ring-gland of Drosophila to a certain extent agrees in location and histology (Nabert 1913) with the corpora allata in different

groups. An embryological study is necessary to decide whether the two organs are homologous.

Summary.—Lethal larvae of the mutation *lgl* in *Drosophila melanogaster* form puparia, but this process is greatly retarded as compared with normal larvae. It is shown that puparium-formation can be accelerated by transplantation of a normal ring-gland to lethal larvae. There is therefore evidence that the ring-gland is an organ of internal secretion and produces a pupation hormone.

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FREQUENCY OF INDUCED BREAKS IN CHROMOSOMES OF DROSOPHILA MELANOGASTER

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One of the effects of x-radiation of living cells is the production of chromosome breaks. When such a break takes place detectable genetic changes frequently occur at the point of breakage. This coincidence is so high that the suggestion has been offered that a break in a chromosome may result from a change in a gene.[1] To test this hypothesis a study has been made of the frequency of breakage of the *Y*-chromosome and the autosomes of *Drosophila melanogaster*. Genetic data suggest that the *Y*-chromosome of this species is essentially inert as compared with the *X*-chromosome and the autosomes. Cytological studies show that the *Y* is heteropyknotic during interphase and prophase stages of mitosis[2] and that it is represented in salivary gland nuclei by a small mass of heterochromatin composed of very few bands.[3] If a change in a gene is responsible for a break in a chromosome or favors the occurrence of such breaks, it is to be expected that the

autosomes with their large genetically active regions would show a considerably greater proportion of breaks than the relatively inert *Y*-chromosome.

Genetic analysis of translocations suggests that the frequency of breaks is less in the *Y*-chromosome than in the *X*-chromosome.[4] Cytological analysis of salivary gland nuclei, however, provides a more rapid and sensitive method of detecting chromosome breaks and has been employed in the present study.

Frequency of Chromosome Breaks.—Determination of the frequency of chromosome breaks recorded here is based on a cytological analysis of the salivary gland chromosomes of 264 male larvae which were the first generation progeny of Oregon-*R* fathers, irradiated at 3000, 4000 or 5000 r-units, and Oregon-*R* or Swedish-*b* mothers. Of the 264 pairs of glands studied, 99 revealed chromosomal alterations and rearrangements. A total of 262 breaks were diagnosed. They were distributed among the chromosome limbs as indicated in table 1. Since many inversions and some deficiencies

TABLE 1

DISTRIBUTION OF BREAKS AMONG THE CHROMOSOMES

CHROMOSOME LIMB	TOTAL BREAKS	INVERSION BREAKS	TRANSLOCATION BREAKS
2*L*	43	7	36
2*R*	77	25	52
3*L*	35	5	30
3*R*	58	10	48
4	5	0	5
Y	44	0	44

occur within the limbs of autosomes, but were not detectable within the material representing the *Y*-chromosome in salivary gland nuclei, the number of translocations is the best index to the relative frequency of breaks in the *Y* and the autosomal limbs. Identification of translocations involving the *Y* has depended to a considerable degree on a comparison of all available nuclei on a slide in order that pairing relationships between translocated sections might be determined statistically. On this basis the 44 rearrangements of *Y*-chromosome material have been diagnosed. There is, however, the possibility that some translocations between the *Y* and the heterochromatic regions of the autosomes were not recognizable. A similar source of error probably exists in the failure to detect exchanges between heterochromatic sections of the autosomes. Such translocations have been observed only rarely in the present study, although their more frequent existence is suggested by the fact that heterochromatic regions of the salivary chromosomes show a higher incidence of breaks than euchromatic regions of equal size.[5] For these reasons the data furnished in table 1 represent approximate rather than absolute values.

The position of these breaks along the autosomes was determined and the data will be presented in another publication together with more extensive data obtained from female larvae.[5] The relative frequency of *Y*-chromosome breaks is somewhat lower than expectancy on the basis of comparative lengths of metaphase chromosomes furnished by Gowen and Gay[6] ($Y = 1$; $2 = 1.2$; $3 = 1.5$), but on the other hand is greater than expectancy on the basis of prophase measurements made at a time when the autosomes are extended and the *Y* is heteropyknotic. Since relatively little is known of the structure of the sperm of *D. melanogaster* and the arrangement of the chromosomes at the time of irradiation, a direct comparison of frequencies is impossible. It is evident, however, that the incidence of *Y*-chromosome breakage is not appreciably lower per unit of mitotic chromosome length than in the autosomes.

Discussion.—From the present study it is evident that chromosome breakage is not a resultant of gene alteration. Not only is the frequency of breakage in the genetically inert *Y*-chromosome approximately equal to that occurring in genetically active regions, but the cytological evidence indicates that breaks occur at various loci in both the long and short limbs of the *Y*-chromosome.

The distribution of chromosome breaks determined by Patterson, Stone, Bedichek and Suche[7] from genetic analysis of x-ray induced translocations indicates that breaks occur most frequently at the free ends or near the spindle fibre attachment regions. The salivary gland analysis reported in the present paper has furnished no evidence that breaks occur more freely at the ends of the chromosomes than in other euchromatic regions, but the autosomes in these nuclei reveal a preponderance of breaks in the heterochromatic regions adjacent to the spindle fibre loci. Accordingly, the question arises whether breakage in heterochromatin is more frequent than in euchromatin. Available evidence suggests that it is not. Breaks in heterochromatin, although more frequent than breaks in euchromatin on the basis of salivary chromosome measurements, are of approximately equal frequency if metaphase lengths are considered. Moreover, a comparison of the incidence of breakage in the *Y*-chromosome and in the autosomes leads to a similar conclusion. If heterochromatin fragmented more easily than euchromatin it would be expected that the heterochromatic *Y*-chromosome would show a much higher proportion of breaks than the autosomes. Since it does not, breakage under the influence of x-rays must be attributed to some property common to all of the chromosomes, irrespective of their division into heterochromatic and euchromatic regions.

Thus, the cytological evidence presented in this paper indicates that the frequency of breaks in the heterochromatic *Y*-chromosome and the essentially euchromatic autosomes is similar per length of metaphase figures. Two possibilities may be considered in interpreting this observation. It

may be assumed, as Muller and Painter[8] previously did, that the breaks are proportional to chromonema length, and that metaphase figures furnish an index of comparative lengths of euchromatic and heterochromatic regions: or it may be assumed, as Muller, Gershenson and Prokofyeva-Belgovskaya[9] are inclined to believe at present, that metaphase figures do not approximate actual lengths of euchromatic and heterochromatic chromonemata and that breaks occur more frequently in heterochromatic than in euchromatic regions of similar length. Our evidence supports the possibility mentioned first. In the present work the position of each breakage point was plotted within the limits of the respective sections of Bridges' salivary chromosome map,[10] which divides each euchromatic arm of the chromosome into 19 regions. These data will be published with more extensive similar data giving a total of about one thousand breaks.[5] They indicate that breaks are distributed at random throughout the euchromatic regions of chromosomes as they appear in salivary glands. The data show an accumulation of breaks in heterochromatic (inert) regions. If, however, the length of inert regions is determined from prophase or early metaphase plates, the number of breaks per unit of length is similar in euchromatic and heterochromatic regions of autosomes, and also in the heterochromatic *Y*-chromosome. This similarity may be accidental. It seems more probable, however, that it is caused by a structural similarity of chromonemata of heterochromatic and euchromatic regions. It seems probable that the metaphase chromosomes approximate more closely than salivary chromosomes the proportional chromonema length of the two regions, and that the frequency of breaks per unit length is the same in both.

If the frequency of breaks per unit length of chromonema is similar in two such different regions as the euchromatic and the heterochromatic, it would indicate that the structure of the chromenema is similar in both. This is equivalent to saying that the structure of the chromonemata is similar throughout the whole length of the chromosome. and this can be extended further to include all chromosomes. Chromosomes then can be visualized as composed of fibre-like chromonemata, which are structurally similar throughout the whole complex, and to which the genes are linked. Such a picture parallels closely the picture of a fibre protein molecule as outlined by Astbury.[11] The backbone of the protein molecule may correspond to the fundamental unit of a chromonema and a group of radicals attached to the backbone may correspond to what we call a gene. In order that such a chromonema may be visible, a lattice structure containing a large number of backbones linked together through radicals would be required. As suggested by Wrinch,[12] basic radicals of protein molecules would form salts with the nucleic acid present in the nucleus and thus the bands visible in salivary gland chromosomes may be indicative of the position and the number of such basic radicals. Consequently, inert re-

gions indicate the dearth of such radicals. Close correspondence observed between bands and loci would require the existence of such close relationship between basic radicals and genes.

Summary.—Salivary chromosome analysis of 262 breaks induced in the sperm indicates that these breaks occur with equal frequency in heterochromatic *Y* and euchromatic autosomes. This is interpreted as indicating that the structure of the chromonema is similar in all regions of chromosomes.

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ON THE DIRECT PRODUCT OF IRREDUCIBLE REPRESENTATIONS OF THE SYMMETRIC GROUP

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The direct product $\Gamma . \Gamma'$ of an irreducible representation Γ of the symmetric group on m letters by an irreducible representation Γ' of the symmetric group on n letters furnishes a (reducible) representation of the symmetric group on $m + n$ letters. We have given previously[1] a method furnishing the analysis of $\Gamma . \Gamma'$ into its irreducible components and have calculated tables giving the results of this analysis for all values of m, n for which $m + n \leqslant 9$. For larger values of m and n the method referred to was somewhat time-consuming and we present here a refinement of it which leads rapidly to the desired result. To illustrate the method we

choose the example $\Gamma = D(4, 3, 1)$ (an irreducible representation of dimension 70 of the symmetric group on 8 letters); $\Gamma' = D(2^2, 1)$ (an irreducible representation of dimension 5 of the symmetric group on 5 letters). $(\Gamma.\Gamma')$ is a reducible representation of dimension 70. 5. 13! ÷ 8! 5! = 450, 450 of the symmetric group on 13 letters. We choose this illustration because its analysis has already been furnished, but incorrectly, by Littlewood and Richardson.[2] Since these authors claim to have checked their result by a comparison of dimensions we must conclude that the incorrect analysis given by them is due to inaccurate copying of the correct result which they undoubtedly possessed. However, they had to confess in their paper that "No simple proof has been found that will demonstrate it" (i.e., the method used by them) "in the general case." The method discovered by us is in detail essentially simpler, and a very simple proof of it follows from the analysis given in our previous paper.

The first step in finding the analysis of the direct product of $\Gamma = D(\lambda_1, \ldots \lambda_\kappa)$ by Γ' is to prefix a λ_1 to every term of the analysis of the direct product of $D(\lambda_2, \ldots \lambda_\kappa)$ by Γ'. Thus we read from the tables in our paper already referred to that

$$D(3, 1).D(2^2, 1) = D(5, 3, 1) + D(5, 2^2) + D(5, 2, 1^2) + D(4, 3, 2) + D(4, 3, 1^2) + 2D(4, 2^2, 1) + D(4, 2, 1^3) + D(3^2, 2, 1) + D(3, 2^3) + D(3, 2^2, 1^2)$$

and so the terms in $\Gamma.\Gamma'$ which start off with a 4 are (we omit, for brevity, the prefix D and observe that $(4, 5, 3, 1) = (4, 5, 2^2) = (4, 5, 2, 1^2) = 0$): $(4^2, 3, 2) + (4^2, 3, 1^2) + 2(4^2, 2^2, 1) + (4^2, 2, 1^3) + (4, 3^2, 2, 1) + (4, 3, 2^3) + (4, 3, 2^2, 1^2)$.

To find the terms which start off with a 5 $(= \lambda_1 + 1)$ we write $\{2^2, 1\}$ as a symmetric function of the variables $z_1, \ldots z_{13}$ (where $s_\kappa = z_1^\kappa + z_2^\kappa + \ldots + z_{13}^\kappa$). The desired expression may be read off from the tables on pp. 475–477 of our previous paper. It is $5 \Sigma z_1z_2z_3z_4z_5 + 2 \Sigma z_1^2z_2z_3z_4 + \Sigma z_1^2z_2^2z_3$ (the necessary coefficients 5, 2, 1 being found in the column headed $(2^2, 1)$ in the table for $n = 5$). The coefficient of z_1 in this expression is $5 \Sigma z_2z_3z_4z_5 + 2 \Sigma z_2^2z_3z_4 + \Sigma z_2^2z_3^2$ and we express this in terms of the characteristics of the symmetric group on $4 = 5 - 1$ letters. Thus, from the table for $n = 4$:

$$\{1^4\} = \Sigma z_2z_3z_4z_5;\ \{2, 1^2\} = 3 \Sigma z_2z_3z_4z_5 + \Sigma z_2^2z_3z_4;\ \{2^2\} = 2 \Sigma z_2z_3z_4z_5 + \Sigma z_2^2z_3z_4 + \Sigma z_2^2z_3^2$$

so that

$$\Sigma z_2z_3z_4z_5 = \{1^4\};\ \Sigma z_2^2z_3z_4 = \{2, 1^2\} - 3\{1^4\};\ \Sigma z_2^2z_3^2 = \{2^2\} - \{2, 1^2\} + \{1^4\}.$$

Hence the coefficient of z_1 referred to is $\{2^2\} + \{2, 1^2\}$. The product of this by $\{3, 1\}$ may be taken from the table on p. 485 of our paper and is $\{5, 3\} + 2\{5, 2, 1\} + \{5, 1^3\} + 2\{4, 3, 1\} + 2\{4, 2^2\} + 3\{4, 2, 1^2\} + \{4, 1^4\} + \{3^2, 2\} + \{3^2, 1^2\} + 2\{3, 2^2, 1\} + \{3, 2, 1^3\}$. Hence the terms starting with

5 in the analysis of $(4, 3, 1).(2^2, 1)$ are $(5^2, 3) + 2(5^2, 2, 1) + (5^2, 1^3) + 2(5, 4, 3, 1) + 2(5, 4, 2^2) + 3(5, 4, 2, 1^2) + (5, 4, 1^4) + (5, 3^2, 2) + (5, 3^2, 1^2) + 2(5, 3, 2^2, 1) + (5, 3, 2, 1^3)$.

To find the remaining terms, namely those starting with a 6, we take the coefficient of z_1^2 in the expression for $\{2^2, 1\}$, i.e., $2 \Sigma z_2z_3z_4 + \Sigma z_2^2z_3$. Expressing this in terms of the characteristics of the symmetric group on $3 = 5 - 2$ letters it appears as $\{2, 1\}$ and the product $\{3, 1\}.\{2, 1\}$ is read from the table for $n + m = 7$ on p. 484 as $\{5, 2\} + \{5, 1^2\} + \{4, 3\} + 2\{4, 2, 1\} + \{4, 1^3\} + \{3^2, 1\} + \{3, 2^2\} + \{3, 2, 1^2\}$ and so the terms beginning with 6 are $(6, 5, 2) + (6, 5, 1^2) + (6, 4, 3) + 2(6, 4, 2, 1) + (6, 4, 1^3) + (6, 3^2, 1) + (6, 3, 2^2) + (6, 3, 2, 1^2)$.

Since $\{2^2, 1\}$ is quadratic in z_i there are no other terms and the complete analysis may be exhibited:

$(4, 3, 1).(2^2, 1) = (6, 5, 2) + (6, 5, 1^2) + (6, 4, 3) + 2(6, 4, 2, 1) + (6, 4, 1^3) + (6, 3^2, 1) + (6, 3, 2^2) + (6, 3, 2, 1^2) + (5^2, 3) + 2(5^2, 2, 1) + (5^2, 1^3) + 2(5, 4, 3, 1) + 2(5, 4, 2^2) + 3(5, 4, 2, 1^2) + (5, 4, 1^4) + (5, 3^2, 2) + (5, 3^2, 1^2) + 2(5, 3, 2^2, 1) + (5, 3, 2, 1^3) + (4^2, 3, 2) + (4^2, 3, 1^2) + 2(4^2, 2^2, 1) + (4^2, 2, 1^3) + (4, 3^2, 2, 1) + (4, 3, 2^3) + (4, 3, 2^2, 1^2)$.

The analysis given by Littlewood and Richardson omitted the terms $(5, 4, 2, 1^2) + (5, 4, 1^4) + (5, 3, 2^2, 1)$. In making the check by dimensions it is convenient to observe that 13×11 is a common factor; on dividing through by this we obtain the check

$$3150 = 36 + 40 + 45 + 240 + 72 + 80 + 84 + 144 + 24 + 120 + 35 + 210 + 180 + 450 + 63 + 81 + 112 + 300 + 144 + 60 + 81 + 180 + 84 + 105 + 60 + 120.$$

The indulgent reader may excuse us for calling explicitly to his attention the complexity of the problems with which nuclear physics has to deal. The symmetric group on 13 letters is a group of order $13! = 6, 227, 020, 800$. We have taken a representation of dimension 450, 450 of this group and have analyzed it into its irreducible components.

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DEGREE OF APPROXIMATION BY POLYNOMIALS—PROBLEM α

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It is the object of the present note to state briefly some recent results on polynomial approximation, the proofs of which will appear elsewhere. Detailed references to the literature will be given at that time.

Let C be a Jordan curve in the z-plane and let $\overline{C}$ denote the closed limited set bounded by C. Let $f(z)$ be analytic in C and continuous in $\overline{C}$. It is known[1] that a sequence of polynomials of respective degrees n, $n = 1, 2, \ldots$, in z exists which converges uniformly to $f(z)$ in $\overline{C}$. The study of the relation between the continuity properties of $f(z)$ on C and the degree of convergence of various sequences of polynomials to $f(z)$ in $\overline{C}$ has been called[2] Problem α.

Let $f^{(p)}(z)$ where p is a positive integer denote the pth derivative of $f(z)$; $f^{(0)}(z) \equiv f(z)$. Let $f(z)$ be analytic in C, continuous in $\overline{C}$, and let $f^{(p)}(z)$ satisfy a Lipschitz condition of order α, $0 < \alpha \leqq 1$, on C, i.e.,

$$|f^{(p)}(z_1) - f^{(p)}(z_2)| \leqq L|z_1 - z_2|^{\alpha}, \quad z_1, z_2 \text{ on } C,$$

where L is a constant independent of z_1 and z_2. (For curves more general than those considered here it is proved by Walsh and the author in a paper soon to appear that if $f(z)$ is analytic in C, continuous in $\overline{C}$, and $f^{(p)}(z)$ satisfies a Lipschitz condition of order α on C, then $f^{(p)}(z)$ satisfies this same Lipschitz condition in $\overline{C}$.) For C an *analytic* Jordan curve John Curtiss[3] has shown that there exists a sequence of polynomials $P_n(z)$ of degree n in z such that

$$|f(z) - P_n(z)| \leqq \frac{M}{n^{p+\alpha}}, \quad z \text{ in } \overline{C},$$

and the author[4] has shown that

$$\left| f(z) - \sum_{\nu=0}^{n} a_\nu F_\nu(z) \right| \leqq \frac{M \log n}{n^{p+\alpha}}, \quad z \text{ in } \overline{C},$$

where M is a constant independent of n and z, and $\sum_{\nu=0}^{n} a_\nu F_\nu(z)$ is the sum of the first $n + 1$ terms of the development of $f(z)$ in the Faber[5] polynomials belonging to C. Curtiss (loc. cit.) extends his results to simultaneous approximation on several mutually exterior sets bounded by analytic Jordan curves. The purpose of this note is to announce some results on Problem α in the case where *C is not analytic.*

To be more explicit let C be a Jordan curve in the z-plane which can be represented parametrically by a function

$$z = z(t), \quad a \leqq t \leqq b, \quad z(a) = z(b),$$

where $z'(t) \neq 0$ and $z''(t)$ exists and satisfies a Lipschitz condition of some order $\delta > 0$. We shall say that C is a curve of Type $D^{2+\delta}$. Let $w = \Phi(z)$ map the exterior of C conformally on $|w| > 1$ so that the points at ∞ in the two planes correspond to each other and denote by C_R, $R \geqslant 1$, the image of $|w| = R$ under the inverse map; $C_1 \equiv C$. We suppose without loss of generality that C contains the point $z = 0$. Let $z_1 = \phi_R(z)$ map $\overline{C}_R$, the closed limited set bounded by C_R, in the z-plane, conformally on $\overline{C}$ in the z_1-plane so that $\phi_R(0) = 0$, $\phi'_R(0) > 0$ (the mapping is not conformal on the boundaries, of course). Under these hypotheses it can be shown by mapping the entire configuration on the unit circle and applying a theorem of Martschenko[6] that

$$|z - \phi_R(z)| \leqq N(R - 1), \qquad z \text{ in } \overline{C}, \tag{1}$$

where N is a constant independent of R and z.

Now let $f(z)$ be analytic in C, continuous in $\overline{C}$ and satisfy a Lipschitz condition of order α, $0 < \alpha \leqq 1$, on C. From inequality (1) and the Lipschitz condition on $f(z)$ which is satisfied in $\overline{C}$ we have

$$|f(z) - f(\phi_R(z))| \leqq L_1(R - 1)^\alpha, \quad z \text{ in } \overline{C}. \tag{2}$$

But $f(\phi_R(z))$ is analytic and uniformly bounded in C_R and it can be shown that for each n, $n = 1, 2, \ldots$, a polynomial $P_n(z)$ of degree n in z exists such that

$$|f(\phi_R(z)) - P_n(z)| \leqq L_2 \frac{|\log (R - 1)|}{R^n}, \; z \text{ in } \overline{C}, \tag{3}$$

where L_2 is a constant independent of R, n and z. By combining inequalities (2) and (3) we have

$$|f(z) - P_n(z)| \leqq L_1(R - 1)^\alpha + L_2 \frac{|\log (R - 1)|}{R^n}, \quad z \text{ in } \overline{C}.$$

Now let $R = 1 + (\log n)/n$ and we obtain

$$|f(z) - P_n(z)| \leqq L_3 \left(\frac{\log n}{n}\right)^\alpha, \quad z \text{ in } \overline{C}, \tag{4}$$

where L_3 is a constant independent of n and z.

We consider next the extension to derivatives. Suppose $f(z)$ is analytic in C, continuous in $\overline{C}$, and $f^{(p)}(z)$ satisfies a Lipschitz condition of order α, $0 < \alpha \leqq 1$, on C. By the same argument as above we obtain inequality (4) with $f(z)$ replaced by $f^{(p)}(z)$. Now we take the right-hand member of (4) as the Lipschitz constant[7] belonging to the function $f^{(p-1)}(z) - Q_{n+1}(z)$

where $Q'_{n+1}(z) = P_n(z)$ and approximate to this function by the above method. By repeated application we can show that for each n, $n = 2, 3, \ldots$, a polynomial $P_n(z)$ of degree n in z exists such that

$$|f^{(p)}(z) - P_n(z)| \leqq L_4 \left(\frac{\log n}{n}\right)^{p+\alpha}, \quad z \text{ in } \bar{C},$$

where L_4 is a constant independent of n and z, p is a positive integer or zero and $0 < \alpha \leqq 1$.

Finally we may approximate several functions defined in mutually exterior regions simultaneously; the method used by Curtiss (loc. cit.) serves to make this extension.

Thus we have the following:

THEOREM. *Let the point set S consist of a finite number of closed regions bounded by mutually exterior Jordan curves $C_1, C_2, \ldots, C_\lambda$, all of Type $D^{2+\delta}$. Let the function $f_k(z)$, $k = 1, 2, \ldots, \lambda$, be analytic in C_k, continuous in $\bar{C}_k$, and let $f_k^{(p)}(z)$ satisfy a Lipschitz condition of order α, $0 < \alpha \leqq 1$, on C_k. Let $f(z) \equiv f_k(z)$ where z is in $\bar{C}_k$, $k = 1, 2, \ldots, \lambda$. Then for each n, $n = 2, 3, \ldots$, there exists a polynomial $P_n(z)$ of degree n in z such that*

$$|f(z) - P_n(z)| \leqq M \left(\frac{\log n}{n}\right)^{p+\alpha}, \quad z \text{ in } S,$$

where M is a constant independent of n and z.

[1] J. L. Walsh, *Interpolation and Approximation*, New York, 1935, pp. 36–37.

[2] W. E. Sewell, *Trans. Amer. Math. Soc.*, **41**, 84–123 (1937).

[3] *Bull. Amer. Math. Soc.*, **42**, 873–878 (1936).

[4] *Bull. Amer. Math. Soc.*, **41**, 111–117 (1935).

[5] *Math. Annalen*, **57**, 389–408 (1903).

[6] A. Martschenko, *Inst. Math. V. Stekloff, Acad. Sci. de l'U. R. S. S.*, **12**, 289–290 (1934). Martschenko states the theorem without proof; however, the result follows from a theorem (unpublished) of Dr. S. E. Warschawski.

[7] This is a modification of a method used by Dunham Jackson, *Theory of Approximation*, New York, 1930, pp. 9–10.

SETS OF INDEPENDENT GENERATORS OF A FINITE GROUP

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A set of independent generators of a group G is a set of operators of G such that they generate G but that no subset of these operators generates G. Such a set can frequently be selected so as to involve various numbers of operators. For instance, when G is the symmetric group on the n letters $a_1, a_2, \ldots, a_n$, it is generated by the set of two permutations composed of the cycle $a_1a_1 \ldots a_{n-1}$ and the transposition $a_{n-1}a_n$. It is also generated by the $n - 1$ transpositions $a_1a_2, a_1a_3, \ldots, a_1a_n$.

Both of these sets are obviously composed of independent permutations. When the order of G is of the form p^m, p being a prime number, it is known that every possible set of independent generators of G involves the same number of operators. The number of operators in a set of independent generators of G can obviously not exceed the number of the distinct prime factors which divide the order of G and when G has a set of independent generators which involves this number of operators various of its properties have been determined recently.[1]

In particular, when the order of G is p^m and the number of operators in a set of its independent generators is m then it is the abelian group of type p^m. When $p > 2$ and the number of operators in a set of independent generators is $m-1$ then G may or may not contain operators of order p^2. In the former case its operators of order p generate a subgroup which is of index p under G. To prove this fact it is only necessary to observe that the ϕ-subgroup of such a group is of order p and that every operator of order p^2 contained in such a group generates this subgroup. If the invariant subgroup generated by the operators of order p contained in G would not be of index p under G then the quotient group of G with respect to this invariant subgroup would be at least of order p^2 and the product of an operator of order p^2 contained in G and any operator of this invariant subgroup would have the same pth power as this operator of order p^2. The product of two operators of order p^2 contained in G would have for the pth power the product of their pth powers. Hence G would involve operators of order p which would not appear in the given invariant subgroup, which is obviously impossible.

When G contains no operators of order p^2 and is not a direct product then m is odd and there is one and only one such group for every odd value of $m > 1$. This group is necessarily non-abelian. When G involves operators of order p^2 then the given invariant subgroup involves either p or p^2 invariant operators. In the former case G involves invariant

operators of order p^2 and is abelian when $m = 2$ but non-abelian whenever $m > 2$. There is obviously one and only one such group for every even value of $m > 0$. When the given invariant subgroup involves p^2 invariant operators then G involves no invariant operator of order p^2 and there is one and only one such group for every odd value of $m > 1$. Hence there results the following theorem: *There is one and only one group of order p^m, p being an odd prime number and $m > 0$ being even, which is not a direct product and has a set of $m - 1$ independent generators; when $m > 1$ is odd there are two and only two such groups.* When m is even the possible group involves operators of order p^2 and when m is odd one of the two possible groups involves such operators. The preceding results are supplementary to those given by the present writer in these PROCEEDINGS, volume 23, pages 234–236.

When G is an abelian group of order p^m which has a set of $m - k$ independent generators, then it is cyclic and $m = k + 1$ since it cannot be a direct product, but when it is non-abelian and k has a given value the possible values of m have no upper limit. A necessary and sufficient condition that a group of order p^m has a set of $m - k$ independent generators is that the ϕ-subgroup of G is of order p^k. When $k = 2$ and the ϕ-subgroup of G is cyclic, this subgroup is generated by some operator of G and is found in the central of G since p is an odd prime number. If it were not generated by an operator of G it would be generated by a commutator of G since it appears in the ϕ-subgroup of G. This is impossible since no operator of G would then be of order p^3 and the pth power of every operator of G would therefore be in the central of G, since every invariant subgroup of a group of order p^m contains invariant operators of order p if its order is at least p.

If such a G involves operators of order p^3 its operators of orders p and p^2 generate an invariant subgroup of G and hence the operators of order p^2 in the ϕ-subgroup are commutative with all the operators of a set of generators of G. That is, *when G has a set of $m - 2$ independent generators and its ϕ-subgroup is cyclic then this subgroup appears in the central of G and the order of the commutator subgroup of G is p unless G is the cyclic group of order p^3.* All the operators of order p contained in G generate a subgroup of index p^2 under G, all of its operators of order p^2 generate a subgroup of index p under G. These theorems result directly from the facts that the order of the commutator subgroup of G cannot exceed p and that p is an odd prime number.

The italicized theorem of the preceding paragraph can easily be extended so as to apply to any group of order p^m having a cyclic ϕ-subgroup of order p^k, p being an odd prime number and G being not a direct product. Such a group cannot be abelian unless $m = k + 1$, and when it is non-abelian it contains operators of order p^{k+1} since the operators of order p^k in the

ϕ-subgroup cannot be commutators arising from two operators whose orders are less than p^{k+1} in view of the fact that the pth power of every operator of G appears in its ϕ-subgroup. The operators of order p^{k+1} contained in G generate G and hence the operators of highest order in the ϕ-subgroup of G are commutative with every operator of a set of generators of G. Therefore it results that if a group of order p^m has $m - k$ independent generators, p being an odd prime and the ϕ-subgroup of G being cyclic, then the commutator subgroup of G is of order p whenever G is non-abelian. In particular, there is one and only one non-abelian group of order p^m which is generated by two of its operators and has a cyclic ϕ-subgroup.

[1] G. A. Miller, *Proc. Nat. Acad. Sci.*, **23**, 333–337 (1937).

ON FUNCTIONAL EQUATIONS IN LINEAR TOPOLOGICAL SPACES[1]

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1. *Introduction.*—A linear topological space is an abstract linear space T: $(x, y, \ldots)$ with real multipliers $\alpha, \beta, \ldots$ which supports a Hausdorff neighborhood topology, such that the fundamental operations of "vector addition" $x + y$ and "scalar multiplication" αx are continuous. These spaces have been studied[2] recently by A. Kolmogoroff, J. v. Neumann, A. Tychonoff, A. D. Michal and E. W. Paxson. Tychonoff generalized the Brouwer-Schauder fixed point theorem to linear topological spaces, and applied this result to obtain an existence theorem for infinite (even non-denumerable) systems of real differential equations. Paxson has used Tychonoff's fixed point theorem in an interesting way in the study of "ordinary" differential equations for functions whose values are in a linear topological space. However, Tychonoff's theorem and hence all the existence theorems flowing from it are "non-effective." In addition, Tychonoff's result tells us nothing about the *uniqueness* of the fixed point. To overcome these difficulties it would be desirable to have an analog for linear topological spaces of the well-known Lipschitz condition, such that the method of successive approximations could be applied under suitable restrictions. We shall see that a satisfactory type of Lipschitz condition can be stated in terms of the sets of a "K-system."

2. *K-Systems.*—If to each element x of a linear topological space T there is ordered a set $K(x)$ subject to the following conditions:

(1) *each* $K(x)$ *is convex;*
(2) $x \epsilon K(x)$ *and the origin* $\theta \epsilon K(x)$; *if* $x \neq \theta$, $\alpha > 1$, *then* $\alpha x \bar{\epsilon} K(x)$;
(3) $K(\alpha x) = \alpha K(x)$ *for* $\alpha \geqslant 0$;
(4) $y \epsilon K(x)$ *implies* $K(y) \subset K(x)$;
(5) *each* $K(x)$ *is bounded;*[3]

then the family of sets $K(x)$ will be called a *K-system* for the space.

In the special case of a linear normed space[4] the sets $K(x) = \{y\}$, $||y|| \leqslant ||x||$ form a K-system. In any linear topological space the closed line segments joining the origin θ to the point x form a K-system $\mathfrak{K}_0$ which is "minimal" in the sense that each set $K(x)$ of an arbitrary K-system contains the corresponding member $K_0(x)$ of the system $\mathfrak{K}_0$ ($K_0(x)$ being merely the line segment αx, $0 \leqslant \alpha \leqslant 1$). We shall see in theorem 2 how K-systems may be generated in a wide class of linear topological spaces. First, however, we show how K-systems may be used in obtaining an existence theorem.

3. *The functional equation* $y = f(y)$.

THEOREM 1. *Let* $z = f(y)$ *be a continuous transformation of* Y *into part of itself, where* Y *is a sequentially complete*[5] *sub-set of a linear topological space* T. *Let* $\{K(y)\}$ *be any K-system with respect to which the function* $f(y)$ *satisfies the "Lipschitz" condition*

$$f(y) - f(z) \subset \mu K(y - z)$$

for all pairs y, z, ϵ Y *where* μ *is a positive constant less than one.*

Then the transformation $z = f(y)$ *of* Y *into part of itself has a unique fixed point.*

PROOF: For any chosen $y_0 \epsilon Y$, define y_ν recurrently by $y_{\nu+1} = f(y_\nu)$, $\nu = 0, 1, 2, \ldots$, so that $y_\nu \epsilon Y$ for all ν. Using properties (3) and (4) of K-systems and an induction we obtain

$$y_{\nu+1} - y_\nu \subset \mu^\nu K(y_1 - y_0), \nu = 1, 2, 3, \ldots$$

Hence we have

$$\begin{aligned} y_{\nu+\rho} - y_\nu &= y_{\nu+\rho} - y_{\nu+\rho-1} + \ldots + y_{\nu+1} - y_\nu \\ &\epsilon \mu^{(\nu+\rho-1)} K(y_1 - y_0) + \ldots + \mu^\nu K(y_1 - y_0) \\ &= (\mu^{(\nu+\rho-1)} + \ldots + \mu^\nu) K(y_1 - y_0), \end{aligned}$$

where the last step is justified since by (1) $K(y_1 - y_0)$ is a convex[6] set. By (5) the set $K(y_1 - y_0)$ is bounded, so that there is a $\delta > 0$ corresponding to each neighborhood U of the origin θ such that $0 < \beta < \delta$ implies $\beta K(y_1 - y_0) \subset U$. Now $0 < \mu < 1$ so that $\sum_{\nu=1}^{\infty} \mu^\nu$ converges, and for sufficiently large ν and any ρ we have $y_{\nu+\rho} - y_\nu \epsilon U$, whence y_ν is a Cauchy sequence. The existence of a fixed point now follows immediately, while the uniqueness

is readily proved by using property (2) together with the inequality $0 < \mu < 1$.

4. *Generation of K-Systems in Locally Convex Spaces.*—We say that the space T is *locally convex* if every neighborhood of the origin θ contains a convex neighborhood of θ. The proof of the next theorem is rather long and will be omitted here.

THEOREM 2. *In any locally convex linear topological space there always exist complete neighborhood systems* $\mathfrak{U}$: $(U, V, W, \ldots)$ *of the origin with the properties*

(i) *if* $U \in \mathfrak{U}$, *then* U *is convex,*

(ii) *if* $U \in \mathfrak{U}$ *then* $\alpha U \in \mathfrak{U}$.

For any such neighborhood system $\mathfrak{U}$ *put*[7] $K(x) = \Pi(U)$. *Then the sets* $K(x)$ *form a K-system for the space* T *and we have*[7] $K(x) = \Pi(\bar{U})$. This theorem gives us a useful way of finding K-systems in any particular locally convex space. For instance in weakly topologized[8] Hilbert space—the theorem tells us that the sets $K(f)$ of all g for which $|(g, \varphi)| \leqslant |(f, \varphi)|$ as φ runs over H, form a K-system.

5. *An existence theorem for a differential equation.*

THEOREM 3. *Let* T *be a locally convex, sequentially complete linear topological space and let* $\mathfrak{U}$ *be a complete neighborhood system with the properties* (*i*), (*ii*) *of theorem 2. Let* I *be a real open interval,* G *an open set of* T. *If the function* $f(\tau, y)$, *defined and continuous for* $\tau \in I$, $y \in G$ *and with its values in* T, *satisfies the "Lipschitz" condition* $f(\tau, y) - f(\tau, z) \subset \mu\Pi(U)$ (μ *a constant* > 0) *for all* $\tau \in I$, $y, z \in G$, *then for any chosen* $\alpha \in I$ *and* $a \in G$ *we may assert that*

(1) *The differential system*

$$\frac{dy}{d\tau} = f(\tau, y), \quad y(\alpha) = a$$

has at most one continuous solution with values in G.

(2) *There exists a* $\delta > 0$ *such that the differential system has a continuous solution* $y = y(\tau)$ *on the closed interval* $[\alpha - \delta, \alpha + \delta]$ *with its values in* G.

By interpreting the space T in different ways a variety of new existence theorems may be established. For instance, on taking T to be the space[9] (s) of infinite sequences $x = (x_1, x_2, \ldots)$ of real numbers, we are led to an existence and uniqueness theorem for an infinite system of real differential equations. Again if T is taken to be a weakly topologized Hilbert space, we have an existence theorem for "weak" differential equations in Hilbert space.

[1] This paper summarizes the results of part III of my dissertation, California Institute of Technology (1937).

[2] A. Kolmogoroff, "Normierbarkeit eines topologischen linearen Raumes," *Studia Math.*, **5**, 29–33 (1934); J. v. Neumann, "On Complete Topological Spaces," *Trans. Am. Math. Soc.*, **37**, 1–20 (1935); A. Tychonoff, "Ein Fixpunktsatz," *Math. Ann.*, **111**, 767–776 (1935); A. D. Michal and E. W. Paxson, "The Differential in Abstract Linear Spaces with a Topology," *Comptes Rendus, Warsaw Acad. Sci.* (in press). See résumé, *Comptes Rendus, Paris Acad. Sci.*, **202**, 1741–1743 (1936); see also forthcoming articles by E. W. Paxson in *Matematiceski Sbornik* and the Paris *Comptes Rendus*.

[3] For three equivalent definitions of boundedness in linear topological spaces see Kolmogoroff, loc. cit., v. Neumann, loc. cit., Michal and Paxson, loc. cit.

[4] For the definition see Banach, *Operations Lineaires*, pp. 26 and 53.

[5] That is, every Cauchy sequence of Y has a limit in Y. Cf. v. Neumann, loc. cit., p. 1.

[6] For a list of properties of convex sets see G. Birkhoff, "Integration of Functions with Values in a Banach Space," *Trans. Am. Math. Soc.*, **38**, p. 359 (1935).

[7] $\prod_{x \in U,\, U \in \mathfrak{U}} (U)$ denotes the intersection of all the sets U of $\mathfrak{U}$ containing x, while $\bar{U}$ denotes the closure of U.

[8] Cf. v. Neumann, loc. cit., p. 15, def. 12 (*b*).

[9] Cf. for example, Banach, loc. cit., p. 10. While (*s*) is metric, the weakly topologized Hilbert space is non-metrizable. Neither space is "normable."

INTERMEDIATES OF VITAMIN B_1 AND GROWTH OF PHYCOMYCES

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In studying the nutrient requirements of excised roots of tomato (*Lycopersicon esculentum*) Robbins and Bartley[1] found a solution of mineral salts, cane sugar and vitamin B_1 to be adequate for unlimited growth. No growth occurs if any one of the three constituents listed is omitted. However, the vitamin may be replaced by a mixture of 2-methyl-5-bromomethyl-6-aminopyrimidine (or 2-methyl-5-ethyoxymethyl-6-aminopyrimidine) and 4-methyl-5-hydroxyethylthiazole,[2] the two intermediates used by Williams and Cline[3] in synthesizing the vitamin. Furthermore, unlimited growth of excised tomato roots occurs in a solution of mineral salts, cane sugar and the thiazole[2] though the growth is less rapid than in a medium containing the vitamin B_1 or both intermediates. The pyrimidine will not replace the vitamin.

If the intermediates replace the vitamin for excised tomato roots can they be used as substitutes for vitamin B_1 for other organisms? A mixture of the pyrimidine and the thiazole referred to above has been found to cure polyneuritis in pigeons.[4] Neither the pyrimidine nor the thiazole

alone is effective. The minimum curative dose of the intermediates is, however, several times the minimum quantity of B_1 which is effective.

Schopfer[5] has reported that *Phycomyces Blakesleeanus* requires vitamin B_1. We have found that a mixture of 2-methyl-5-bromo-methyl-6-amino-pyrimidine (or the ethyoxypyrimidine) and 4-methyl-5-hydroxyethyl-thiazole is as effective as molecularly equivalent amounts of B_1 in determining the dry matter produced by *Phycomyces* in a period of two weeks. Neither the thiazole nor the pyrimidine alone is effective.

The thiazole contains reduced sulphur. Can other sulphur compounds be substituted for the thiazole? Such a possibility is suggested by the report by Volkonsky[6] that *Saprolegnia sps.* require reduced sulphur and are unable to utilize sulphur in the oxidized form. Negative results, however, were secured with *Phycomyces* by the authors when the following sulphur compounds were substituted for the thiazole in a modified Coon's medium (0.5% $MgSO_4$, 7% H_2O, 1.5% KH_2PO_4, 10% asparagin, 10% dextrose) and the pyrimidine: *dl*-methionine, glutathione, thioglycolic acid, S-diphenyl-thiourea, thiopropionamide, allylthiourea, thiobarbituric acid, thiourea, 2-amino-4-methylthiazole hydrochloride, 2-amino-4-(p-diphenyl) thiazole, 1-chlorobenzothiazole, 1-mercaptobenzothiazole, 1-phenylbenzothiazole, 1-methylmercaptobenzothiazole (1-thiomethyoxybenzothiazole) and 5-amino-1-mercaptobenzothiazole hydrochloride.

Negative results were secured also when ethylene chlorhydrin or pimelic acid was substituted for vitamin B_1. Ethylene chlorhydrin is effective in breaking dormancy of plants[7] and pimelic acid has been reported to be an accessory growth factor for a strain of the diphtheria bacillus.[8]

Pyrimidine compounds are included in the hydrolytic products of nucleic acid. Will nucleic acid replace the pyrimidine? We have not succeeded in cultivating *Phycomyces Blakesleeanus* in the modified Coon's solution and thiazole[9] to which nucleic acid or the acid hydrolysate of nucleic acid was added.

It appears from these results and those reported earlier that the pigeon and *Phycomyces* differ from tomato roots in requiring either vitamin B_1 or both intermediates. The tomato roots will grow indefinitely in a solution of mineral salts, cane sugar and the thiazole though the growth is less vigorous than when B_1 or both intermediates are supplied. The pigeon and *Phycomyces* differ in that B_1, under the conditions of our experiments, is considerably more effective than equivalent amounts of the two intermediates for the pigeon but equally effective for *Phycomyces*.

It appears that there are heterotrophic organisms capable of continued growth in a medium lacking B_1. We have grown *Aspergillus niger* through six generations in a liquid medium containing mineral salts and pure cane sugar. The dry weight of the sixth generation was approximately equal to that of the first and spore production was normal. On this medium

Phycomyces will not grow unless B_1 or its intermediates are present. Since, in making the transfers of the *Aspergillus*, a few spores only were used in inoculating the new culture media the B_1 present in the original spores must have been fractionated to an inappreciable amount in the 6 successive transfers.

There are organisms (if we may refer to excised tomato roots as organisms) which require thiazole for continued growth. There are others, for example *Phycomyces Blakesleeanus* and the pigeon, which require vitamin B_1 or both intermediates. Are there organisms for which the pyrimidine alone is adequate? On this question we have no evidence.

Is the vitamin essential or do each of the intermediates function independently? When *Phycomyces* grows in the presence of the intermediates is vitamin B_1 synthesized or does the thiazole perform one essential function and the pyrimidine another? When the organism grows in the presence of the vitamin is the vitamin split into the intermediates which then function or does the entire B_1 molecule act in conditioning growth? The authors are of the opinion that the vitamin is required. This conclusion is suggested as the result of experiments on *Phycomyces* which show that the two intermediates are probably used in molecularly equivalent quantities. The organism was grown in the modified Coon's solution and the two intermediates. In each mixture of the intermediates one was supplied in small amount, the other in considerably larger amount. For each combination in which the thiazole was furnished in small amount and the pyrimidine in larger amount a similar mixture was prepared in which the quantities of the thiazole and pyrimidine were reversed. This will be made clear from the following table in which the dry weights of *Phycomyces* are given as determined at the end of two weeks' growth. The fungus was grown in 20 cc. of modified Coon's solution and a mixture of the thiazole and pyrimidine as given in the table.

AMOUNT IN 10^{-9} MOLE		DRY WEIGHT,
THIAZOLE	PYRIMIDINE	MGMS.
0.01	10.0	9
10.0	0.01	5
0.1	10.0	25
10.0	0.1	26
0.3	10.0	49
10.0	0.3	50
1.0	10.0	115
10.0	1.0	121
3.0	10.0	318
10.0	3.0	320
10.0	10.0	500
10×10^{-9} mole B_1		520

It appears that the dry weight formed in a medium containing thiazole and pyrimidine is determined by the one which is present in smaller concentration and is the same for equal limiting molecular concentrations of either the thiazole or the pyrimidine. For example, with 0.3×10^{-9} mole of thiazole and 10.0×10^{-9} mole of pyrimidine the yield was 49 mgms. and with 0.3×10^{-9} mole of pyrimidine and 10×10^{-9} mole of thiazole the yield was 50 mgms. Other pairs of combinations of the two intermediates show similar results. If the thiazole and pyrimidine functioned independently it would be expected that more growth would be secured in one of a pair of concentrations such as are given in the table. The fact that the two intermediates appear to be used by the organism in molecularly equivalent quantities is best explained by assuming that the vitamin is required and is synthesized from them by the organism.

Since the completion of the experiments on *Phycomyces* summarized here the writers' attention was called to a note by Schopfer and Jung.[10] They find that in a mixture of 4-amino-5-amino-methyl-2-methyl pyrimidine[11] and 4-methyl-5-hydroxyethylthiazole at amounts of 0.01 to 0.4 gamma per 25 cc. the development of *Phycomyces* is identical with that obtained with vitamin B_1. Our results confirm and extend those of Schopfer and Jung.

[1] William J. Robbins and Mary A. Bartley, *Sci.*, **85**, 246–247 (1937).

[2] William J. Robbins and Mary A. Bartley, *Proc. Nat. Acad. Sci.*, **23**, 385–388 (1937).

[3] R. R. Williams and J. K. Cline, *Jour. Am. Chem. Soc.*, **58**, 1504–1505 (1936).

[4] William J. Robbins, Mary A. Bartley, A. G. Hogan and L. R. Richardson, *Proc. Nat. Acad. Sci.*, **23**, 388–389 (1937).

[5] W. H. Schopfer, *Ber. Deutsch. Bot. Gesell.*, **52**, 308–313 (1934).

[6] Michel Volkonsky, *Ann. l'Inst. Past.*, **50**, 703–730 (1933).

[7] F. E. Denny, *Amer. Jour. Bot.*, **13**, 386–389 (1926).

[8] J. Howard Mueller, *Sci.*, **85**, 502–503 (1937).

[9] When the terms, thiazole or pyrimidine, are used in this paper without other qualification the 4-methyl-5-hydroxyethylthiazole or the 2-methyl-5-bromo-methyl-6-aminopyrimidine is meant.

[10] William H. Schopfer and Albert Jung, *Compt. Rend. Acad. Sci. Paris*, **204**, 1500–1502 (1937).

[11] This pyrimidine is identical with the one we have used except that an amino group has been substituted for the bromine.

MECHANISM OF SYMBIOTIC NITROGEN FIXATION. II. THE pO_2 FUNCTION*

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Although there have been numerous suggestions regarding the mechanism of nitrogen fixation through association of leguminous plants and the root nodule bacteria (*Rhizobium sp.*), evidence in support of any particular mechanism is extremely meager.[4] Difficulties encountered in development of successful techniques for attack on the problem undoubtedly account for the lack of pertinent data. In a previous report it was suggested that application of the methods and point of view of physical chemistry might be of value in securing information concerned with the characteristics of the responsible enzyme systems and in providing suitable criteria for selection among several alternative hypotheses. As some of the proposed mechanisms involve molecular oxygen, it is of interest to determine how the fixation process reacts to changes in the partial pressure of oxygen gas in the atmosphere.

Methods. Red clover (*Trifolium pratense*) was grown in a closed system on a nitrogen-poor sand, supplied with all necessary plant nutrients except combined nitrogen and furnished an atmosphere of any desired pO_2. In certain experiments 2-liter flint glass bottles were used as the plant container, and the atmospheres were supplied continuously by means of a flowmeter arrangement.[6] In other experiments the plant containers were 10-liter Pyrex pressure bottles in which an atmosphere of a given pO_2 was prepared by evacuation followed by addition of known quantities of the necessary gases; such atmospheres were renewed every third day. Details of the methods for preparation of cultures, control of environment and harvest of plants are given in previous reports.[3,5] In each experiment one series of plants was inoculated with an efficient strain of *Rh. trifolii* and a parallel series left uninoculated but supplied periodically with NH_4NO_3 as a source of nitrogen.

Results from typical experiments are summarized in tables 1 and 2. Examination of these data together with those from similar experiments lead to the following conclusions in regard to the pO_2 function of the symbiotic nitrogen fixation process in red clover.

1. If the pO_2 is greater than 0.1 atm. but no more than 0.4 atm., formation of dry weight and uptake of *either free or combined nitrogen* is essentially *independent* of the pO_2 of the atmosphere (table 1). It is possible that a maximum obtains in this region of the *pO_2-nitrogen uptake* curve, but our experiments indicate that, if such exists, its position varies with other environmental conditions, especially those concerned with rate of growth of the host plant, e.g., temperature and light. Apparently between partial pressures of oxygen of approximately 0.1 and 0.4 atm. the influence of the pO_2 of the atmosphere on the fixation process is secondary in comparison with other factors so that this region of the pO_2 function may be regarded as a plateau.

2. If the pO_2 of the atmosphere is increased to a point greater than about 0.4 atm., a marked decrease in the uptake of either free or combined nitrogen results. The exact position of the "break" in the curve varies with environmental conditions. Thus in Experiment II (table 1) the plants were grown in the greenhouse during a hot summer;

TABLE 1

EFFECT OF pO_2 ON FIXATION OF NITROGEN BY RED CLOVER

(High pO_2 Experiments)

PO_2 IN ATMOSPHERE ATM.	INOCULATED SERIES DRY WT. MGM.	INOCULATED SERIES TOTAL N MGM.	INOCULATED SERIES % N	NH_4NO_3 SERIES DRY WT. MGM.	NH_4NO_3 SERIES TOTAL N MGM.	NH_4NO_3 SERIES % N
		Expt. I. (*Mar. 29 to May 23, 1932*)				
0.10	520	10.08	2.25	...	23.30	....
0.20	448	10.66	2.38	851	22.90	2.70
0.60	305	7.56	2.48	564	16.90	3.00
		Expt. II. (*June 4 to Aug. 1, 1932*)				
0.10	358	8.12	2.80	617	23.76	3.94
0.20	285	6.82	2.52	386	21.33	5.65
0.40	153	5.09	3.37	270	16.54	6.19
0.60	161	4.77	3.00	272	14.84	6.39
		Expt. III. (*Sept. 3 to Nov. 16, 1932*)				
0.10	563	13.61	2.42	613	19.61	3.20
0.20	679	12.54	2.62	584	18.86	3.23
0.40	466	11.72	2.52	560	19.18	3.42
0.60	405	11.44	2.83	439	15.66	3.57
		Expt. IV. (*Oct. 19 to Dec. 11, 1933*)				
0.20	1050	25.2	2.40	..	24.6	..
0.40	1130	28.0	2.47	1200	34.5	2.87
0.60	300	8.2	2.73	310	10.6	3.41

Figures in all tables on 10-plant basis.

pN_2 = 0.40 atm. in each case; hydrogen to 1.0 atm.

Expt. I. 64-oz. bottles 21 days under pO_2 indicated. 2.50 mgm. nitrogen in plants of inoculated series and 3.75 mgm. nitrogen in plants of NH_4NO_3 series, when placed under indicated pO_2.

Expt. II. 64-oz. bottles 36 days under indicated pO_2. 1.55 mgm. N in inoculated series; 5.55 mgm. N in NH_4NO_3 series.

Expt. III. 64-oz. bottles 23 days under indicated pO_2. 2.25 mgm. N in inoculated series; 3.35 mgm. N in NH_4NO_3 series.

Expt. IV. 10-liter bottles 24 days under indicated pO_2. 3.00 mgm. N in inoculated series: 3.55 mgm. in NH_4NO_3 series.

at the elevated temperatures encountered (30° to 40°C.) assimilation of free as well as combined nitrogen decreased as the pO_2 of the atmosphere was raised from that of normal air (0.2 atm.) to 0.4 atm. In fact, there was some indication that a decrease in the pO_2 from that in the air to 0.1 atm. may cause an *increase* in the quantity of nitrogen fixed at these high temperatures. In experiments made at more normal temperatures, however, plants grown under a pO_2 of 0.4 atm. frequently showed no

evidence of decreased assimilation of nitrogen; hence, the critical portion of the curve under ordinary conditions lies between 0.4 and 0.6 atm. The important point is that plants using combined nitrogen made a response to increases in pO_2 similar to that of plants using free nitrogen independent of the other environmental conditions.

The response to the higher pO_2 seems to be definitely related to increased respiration of the plant with a consequent depletion of carbohydrate reserve. Plants developing under the high partial pressures of oxygen became pale green in color, spindly and smooth, whereas those grown at a more normal pO_2 were dark green, thrifty and pubes-

TABLE 2

EFFECT OF pO_2 ON FIXATION OF NITROGEN BY RED CLOVER

(Low pO_2 Experiments)

PO_2 IN ATMOSPHERE ATM.	INOCULATED SERIES DRY WT. MGM.	TOTAL N MGM.	% N	NH_4NO_3 SERIES DRY WT. MGM.	TOTAL N MGM.	% N
		Expt. V. (*April 21 to June 12, 1933*)				
0.012	244	6.02	2.47	326	14.70	4.51
0.025	341	8.45	2.48	341	15.35	4.50
0.05	380	9.12	2.40	668	22.60	3.38
0.10	479	11.50	2.40	932	23.95	2.57
		Expt. VI. (*June 3 to July 28, 1933*)				
0.012	175	3.83	2.19	304	11.27	3.71
0.025	280	6.28	2.24	462	13.40	2.91
0.05	510	11.10	2.18	612	16.95	2.77
0.10	742	15.80	2.13	770	18.80	2.44
		Expt. VII. (*Dec. 14, 1933 to Feb. 6, 1934*)				
0.012	424	5.90	1.39	362	7.06	1.95
0.025	573	10.20	1.78	700	12.74	1.82
0.05	718	15.15	2.11	..	...	..
0.10	772	16.84	2.18	1280	40.10	2.19
0.20	770	18.32	2.38	..	...	..

pN_2 = 0.40 atm. in Expt. V and VI; 0.80 atm. in Expt. VII; hydrogen to 1.0 atm.

Expt. V. 64-oz. bottles. 24 days under indicated pO_2 1.50 mgm. N in plants of inoculated series and 3.20 mgm. N in plants of NH_4NO_3 series when placed under pO_2 indicated.

Expt. VI. 64-oz. bottles. 32 days under indicated pO_2. 1.35 mgm. N in inoculated series; 3.85 mgm. N in NH_4NO_3 series.

Expt. VII. 10-liter bottles. 29 days under indicated pO_2. 2.55 mgm. N in inoculated series; 5.10 mgm. N in NH_4NO_3 series.

cent. Plants grown under a pO_2 of 0.6 atm. were consistently higher in *percentage* nitrogen (indicative of low carbohydrate) than were the others in spite of a marked decrease in the *absolute* quantity of nitrogen assimilated.

In preliminary experiments it was observed that if the plants were placed under the higher partial pressures of oxygen immediately after germination, they became very fragile and frequently perished in a short time. To prevent the ill effects of this initial "fast living," all the plants were grown for a period in air (CO_2 content raised to 0.5%) until the nitrogen fixation process in the inoculated plants was well under way. This preliminary treatment should be kept in mind in interpreting the results, since it is

apparent that the actual value of dry weight formed and nitrogen assimilated will depend in part on the stage of development of the plants when placed under the indicated pO_2. Thus a plant high in carbohydrate may assimilate considerable free or combined nitrogen under a high pO_2 before the carbohydrate supply is so depleted that restriction of nitrogen assimilation results.

3. Assimilation of nitrogen and formation of dry weight substance decrease with the pO_2 when the latter is less than 0.1 atm. (table 2). The effect of the low pO_2 appears to arise from a general slowing up of the metabolism of the entire plant since there is no change in the *percentage* nitrogen in the plant, i.e., the relation of carbohydrate material to nitrogen remains constant. It is of interest that clover plants kept under a pO_2 of only $^1/_{16}$ that of normal air grew and fixed appreciable quantities of nitrogen.

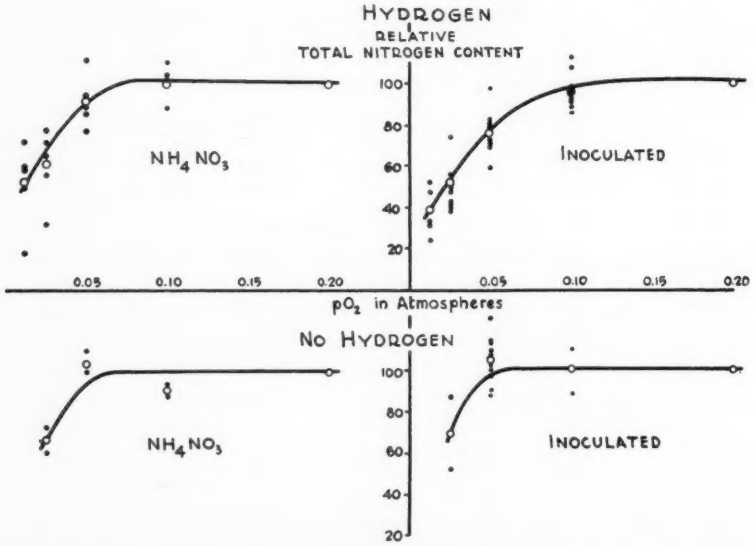

FIGURE 1

Effect of low partial pressures of oxygen on uptake of free and combined nitrogen in the presence and absence of hydrogen. (Full dots refer to individual experiments; circles to the mean of all experiments.)

In all the experiments reported in the first two tables, the pN_2 of the atmosphere was maintained at 0.4 atm., and hydrogen gas added to bring the total pressure to unity. Later studies gave evidence that under these conditions hydrogen may not be an inert gas but will act as a specific inhibitor of the symbiotic nitrogen fixation process. Because of this latter finding it was believed advisable to confirm the essential features of the *pO_2-nitrogen assimilation* function in the absence of hydrogen.

Results of a typical experiment are given in table 3. These data with those from similar experiments lead to the conclusion that in addition to

greater fixation of free nitrogen, the pO_2 function in the absence of hydrogen differs from that in its presence in two respects. Firstly, there is an indication that the "critical" point in the higher range of pressures may come at a slightly lower pO_2 in the absence of hydrogen. An atmosphere of pO_2 of 0.2 atm. would have a pH_2 of 0.4 atm. whereas one with a pO_2 of 0.4 atm. would have a pH_2 of only 0.2 atm. if the pN_2 is kept constant at 0.4 atm. Increase in the pO_2 would tend to lessen the quantity of nitrogen fixed, but decrease in the pH_2 would favor fixation of the free element; hence, in the presence of hydrogen (with a constant pN_2 in the atmosphere) the effect of increasing the pO_2 may be partially masked. Differences in the position of the critical point in the curve due to the effect

TABLE 3

EFFECT OF pO_2 ON FIXATION OF NITROGEN BY RED CLOVER IN PRESENCE AND ABSENCE OF HYDROGEN GAS

PO_2 IN ATMOSPHERE ATM.	INOCULATED SERIES DRY WT. MGM.	INOCULATED SERIES TOTAL N MGM.	INOCULATED SERIES % N	NH_4NO_3 SERIES DRY WT. MGM.	NH_4NO_3 SERIES TOTAL N MGM.	NH_4NO_3 SERIES % N
		Expt. VIII (Jan. 6 to Feb. 19, 1936)				
No hydrogen added						
0.60	182	4.30	2.36	120	4.12	3.45
0.30	405	9.72	2.40	480	11.75	2.45
0.15	400	6.88	1.72	550	13.30	2.41
0.05	500	9.05	1.81	530	12.90	2.43
0.025	242	4.36	1.81	310	8.07	2.60
Hydrogen added						
0.60	135	2.97	2.20	185	6.01	3.24
0.30	235	4.21	1.79	475	10.20	2.15
0.15	...	..	..	655	12.25	1.87
0.05	294	2.94	1.00	495	10.20	2.10
0.025	231	2.24	0.97	400	7.00	1.75

pN_2 = 0.2 atm. in every case; hydrogen added to 1.0 atm. in one series; in other series no gas added (plant grown under partial vacuum).

of hydrogen, however, are probably secondary in comparison with other environmental factors which affect this position.

Secondly, there is definite suggestion that the critical point in the lower range occurs at about 0.05 atm. in the absence of hydrogen rather than at 0.1 atm. which was found in the presence of this gas. More important, however, this difference appears to be characteristic only of the plants of the inoculated series indicative of an effect concerned specifically with the fixation reaction. In order to investigate this possibility more completely a large number of experiments were made in which fixation in the presence and absence of hydrogen at a pO_2 in the vicinity of 0.05 atm. was determined. A summary of the results of all experiments which included the

range of pO_2 less than 0.2 atm. is given in figure 1. The data from the various experiments were combined into one figure by calculation of the *relative* nitrogen content of the plants of each treatment using those grown under a pO_2 of 0.2 atm. as the standard—equal to 100. In some tests the highest pO_2 used was 0.1 atm. and then the plants of this series were used as the standard.

The curves shown in the figure are essentially identical in form except for that of *inoculated plants grown in the presence of hydrogen.* For these plants the nitrogen content decreases significantly as the pO_2 is decreased from 0.1 atm. to 0.05 atm.; the relative nitrogen content of plants grown under the latter pO_2 is only 76 (average of 8 experiments) when compared with the nitrogen content of plants grown under a pO_2 of 0.2 atm. The corresponding values for plants of the other series kept under a pO_2 of 0.05 atm. are: *Inoculated no hydrogen,* 106 (average of 10 experiments); *NH_4NO_3 hydrogen,* 92 (average of 5 experiments); and *NH_4NO_3 no hydrogen,* 103 (average of 3 experiments). It is doubtful if any of these three values is significantly different from 100, but the value, 76, for the *inoculated hydrogen* series indicates a decrease in quantity of nitrogen fixed which is beyond errors of the experiments. Such a finding suggests that the inhibition of nitrogen fixation by hydrogen may be related to an effect on the oxidative processes in the plant. This point is under further investigation in connection with studies dealing with the nature of hydrogen inhibition.

Summary.—Comparison of the pO_2 function of red clover plants fixing elemental nitrogen with that of plants assimilating combined nitrogen indicates that the functions are essentially similar, which leads to the conclusion that molecular oxygen is not *directly* concerned in the symbiotic nitrogen fixation process in these plants. Accordingly, all proposed mechanisms involving molecular oxygen will have to be discarded. Oxygen, however, is important for the fixation reaction in an *indirect* manner, especially as it influences the carbohydrate metabolism of the host plant.[2] This indirect effect is probably the basis of the finding of Virtanen and v. Hausen[1] that oxygen plays a rôle in the process. Evidence is presented that inhibition of symbiotic nitrogen fixation by hydrogen may be connected with effects on the oxidative mechanisms in the host plant.

* Herman Frasch Foundation in Agricultural Chemistry, Frasch Paper No. 141.

[1] Virtanen, A. I., and v. Hausen, S., *Jour. Agr. Sci.*, **26**, 281–287 (1936).
[2] Wilson, P. W., *Wis. Agr. Expt. Sta. Res. Bull.*, **129**, 40 pp. (1935).
[3] Wilson, P. W., *Jour. Amer. Chem. Soc.*, **58**, 1256–1261 (1936).
[4] Wilson, P. W., *Bot. Rev.* **3**, 365–399 (1937).
[5] Wilson, P. W., and Georgi, C. E., *Bot. Gaz.*, **94**, 346–363 (1932).

ON THE LAW FOR MINIMAL DISCRIMINATION OF INTENSITIES. II.

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1. A simple relationship exists between the mean magnitude of the least discriminable interval of intensity (ΔI_m) and the dispersion (σ_{I_2}) of the measurements from which ΔI_m is obtained. The two quantities are in direct proportion.[1] The rule holds when fixed intensities I_1 are compared with adjusted intensities $\tilde{I}_2$, giving $\tilde{I}_2 - I_1 = \Delta I$, and also when an intensity I_1 is progressively changed to a just detectable different level, $\tilde{I}_2$. Two important questions arising involve (1) the manner in which $\sigma_{\Delta I}$ ($= \sigma_{I_2}$) may be held to determine[1] ΔI_m (rather than the reverse) and (2) the nature of the variation measured by $\sigma_{\Delta I}$. The present note is concerned with the second of these points.

The customary treatment of variation in data of this kind tends to regard it as in the class of "errors of measurement," and not uncommonly with the further assumption that the mean error or σ_1 should therefore be *expected* to be proportional to the average value of the quantity estimated or at least to increase with it. There is, of course, no real basis whatever for such a view, theoretical or otherwise. In opposition to this general notion is the conception which holds[2] that under good conditions of experimental control the variation of I_2 is due primarily to the fluctuating reactive capacity of the organism tested. While in a sense realization of this is certainly not completely new,[3] its importance for the investigation of sensory properties has been little understood and is yet to be fully developed. The relation of this variation to additional variables provides means of deciding to what extent it is due to extraneous fluctuation of instrumental or methodological origin and to what degree it is upon the other hand an expression of intrinsic properties of the reacting organism.[9] Variability, taken as the capacity to vary performance,[2] may thus be studied with the expectation of establishing its significant properties.

2. In determinations of differential sensitivity for excitation (somesthetic pressure) by mechanical pressure on the skin, it is possible to vary independently, among other things, the area (A) exposed to excitation and the weight (W) exerting the pressure. When area of application is "constant"—disregarding the changes of area due to progressive deformation of the integument as a function of the applied load—ΔW is found to be related to W in the usual way. The data of Stratton[5] and other experiments[6] show that ΔW decreases with W, log ΔW being an approximately rectilinear function of log W_2. Also, ΔW_m is directly pro-

portional to the variation of W_2. (Since the distribution of $\tilde{W}_2$ for fixed W_1 is essentially "normal," as for $\tilde{I}_2$ in other intensity discrimination tests, the variation may be measured equally well by $A.D._{\Delta W}$ or by σ_{W_2}. Stratton's figures[5] are given in terms of $A.D.$) Figure 1 gives one example. The same kind of relationship holds when *rate of application* of W_2 is the variable (cf. data in [5]). The simple proportionality between $A.D._{\Delta W}$ and ΔW_m is not disturbed when $-\Delta W$ is measured instead of $+\Delta W$, nor when different reference end-points are used.

In these cases W/A = mechanical pressure P, where P has the signifi-

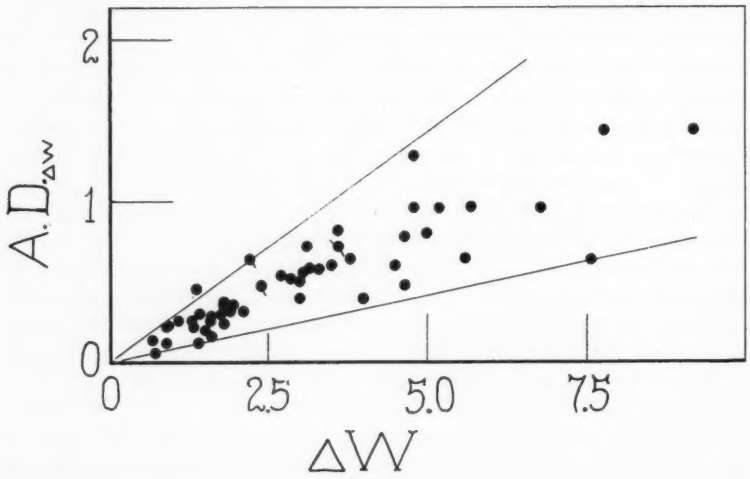

FIGURE 1

Data from Stratton,[5] one subject ("*A*"); $n = 10$; relation between $A.D._{\Delta W}$ and ΔW "constant" area of application of weight (overlapping points indicated by tags). Data on other observers are similar. On a log-log grid the dispersion of the points of course would be confined within parallel lines; the vertical span at any level of $\Delta W = const. \times mean\ A.D._{\Delta W}$ (cf. Fig. 2).

cance of intensity I, and W and P are in direct proportion to one another if the area of application A is "constant." With tests of kinaesthesis, P or its homolog in intensity *per* unit area or *per* unit volume of muscle under tension may be altered by certain procedures.[7] Thus P is in effect *decreased* when test-weights are lifted by the forearm instead of from the wrist; ΔP, therefore, is found less when the same mass W_1 is lifted by the forearm and compared with $\tilde{W}_2$, as shown by the fact that ΔW is then less.[6] Moreover, if W_1, lifted from the wrist, and W_2, lifted from the elbow, are compared in succession or simultaneously, ΔW is less than when the two are similarly compared by lifting from the wrist alone. "P" is the

same, however, if W_1 and $\tilde{W}_2$ are simultaneously lifted one by each arm and in the same manner, but ΔW is then less than it is for successive tests with the same hand;[7] the peripheral area affected has been doubled. In all of these instances, however, and in other elaborations of such comparisons, ΔW and $\sigma_{\Delta W}$ are in the same direct proportion.[5,6,7]

Relationships of this sort, of course, are to be found clearly only in homogeneous data.[1] Their use in terms of numbers of excitation units involved and their importance for the question of *number of excited elements versus* the *proportion* of these to the totality of potentially excitable elements in a given peripheral area we shall return to in a subsequent connection. Our purpose here is to utilize facts of this order for an examination of the nature of $\sigma_{\Delta W}$. The other problems mentioned are better approached by means of experiments with vision and perhaps audition where precise tests are possible over a much wider range of intensities.

For auditory excitation[8] ΔI is less for binaural excitation by I_1 than for monaural; the peripheral "area" is increased by binaural stimulation, but ΔI_m and $\sigma_{\Delta I}$ are in direct proportion. With visual discriminations I corresponds to P. When the excited retinal area is increased, with I_1 constant, ΔI is decreased.[10]

Similarly, the critical illumination at given flicker frequency is less ($I_m - 0 \equiv \Delta I$) when the area is increased.[12] The correspondence between I_c ($\equiv \Delta I$) and I is not disturbed by altering area.[12] Hence we find here also, as well as in measurements of visual intensive discrimination, that ΔI and $\sigma_{\Delta I}$ decline when area is increased. The fact that not total energy of illumination but I is the quantity measured shows that "errors" in the adjustment of I_2 in such experiments can scarcely be responsible for the correlation between ΔI and $\sigma_{\Delta I}$. Moreover, in the cone portion of the flicker curve for vertebrates, σ_{I_2} ($\equiv \sigma_{\Delta I}$) is not simply proportional to I_2 but is approximately a power function of it with exponent < 1.[12]

3. It has been of interest to examine the behavior of ΔP and of $\sigma_{\Delta P}$ as a function of P for somesthetic pressure, since additional evidence on this point is obtained without too great difficulty. We shall deal here only with the matter of variation. Additional details concerning the experiments are given in another place.[4] When area of application is varied, the total mass W_1 must be changed appropriately to maintain P_1 constant. W_1 and area A must then be kept in direct proprotion. But inasmuch as for increasing area A, for P_1 fixed, it is found that ΔP and also $\sigma_{\Delta P}$ decline, the relationship between W_1 and ΔP is the *reverse* of that between W_1 and ΔW when A is constant. In the latter case, ΔW and $\sigma_{\Delta W}$ increase with W_1; in the former, ΔP and $\sigma_{\Delta P}$ decrease. Since $\sigma_{\Delta W}$ ($= \sigma_{W_2}$), with P_1 fixed, passes through a maximum as A is increased, it cannot be held that the proportionality between ΔP and $\sigma_{\Delta P}$ is due to "experimental errors" in the measurement of W_2. The errors involved in the adjustment of

W_1 and of W_2 include "errors" of weighing and of estimating A. Both groups of errors should increase in general with increase of A. The fact that $\sigma_{\Delta P}$, where $\Delta P = \Delta W/A$ for fixed P_1, *declines* systematically as A is made greater makes it absurd to suppose that $\sigma_{\Delta P}$ is determined primarily by errors in the physical measurement of A or W.

While in these experiments (Fig. 2) it is still true that ΔW and $\sigma_{\Delta W}$ are directly in proportion, this is shown not to be dependent upon an increase of W_2 with increase of ΔP (or ΔW). Consequently, the fact that $\sigma_{\Delta W}$ increases

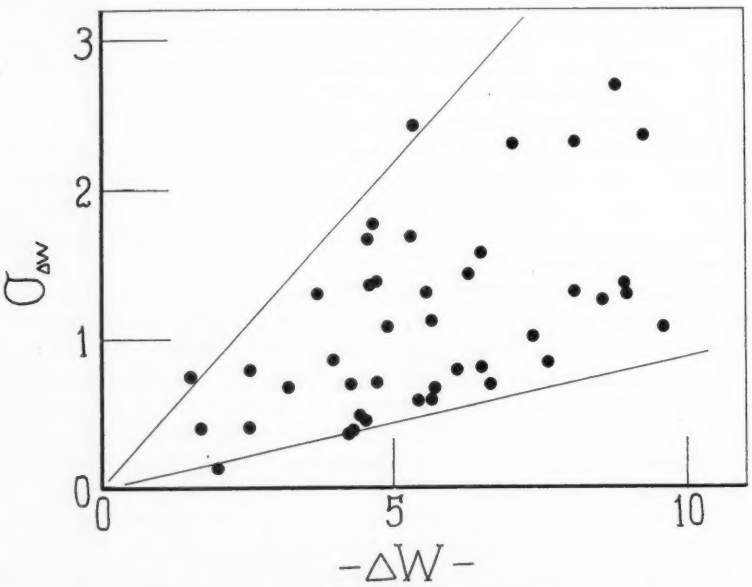

FIGURE 2

The data of the present tests in terms of ΔW and $\sigma_{\Delta W}$; $W_1/A = P_1 =$ *const.* Compare with figure 1. (The positions of the points are not the same as in figure 2 since as area A is increased ΔW passes through a maximum.)

with W_1 when A is constant is not to be ascribed to simple physical "errors" in the successive adjustments of W_2. It must then originate in the organism (say) in the organism's fluctuating capacity to execute the discrimination by which P_2 is reacted to as just noticeably greater than P_1. The fact that widely different procedures involving sensory discrimination of mechanically induced excitation give a characteristic uniformity in the coefficient of variation for ΔP is additional evidence that the variability concerned is basically a specific property of the organism itself.

4. Figure 3 shows the relation between ΔP and $\sigma_{\Delta P}$ for somesthetic pressure. Each point is based upon ten measurements with one subject (A.H.H.) during six successive days. The area studied was located about one-third the distance from the elbow to the wrist on the volar aspect of the left forearm. Cylindrical glass vessels drawn thin were constructed with different bottom areas. Water was added from a burette at the rate

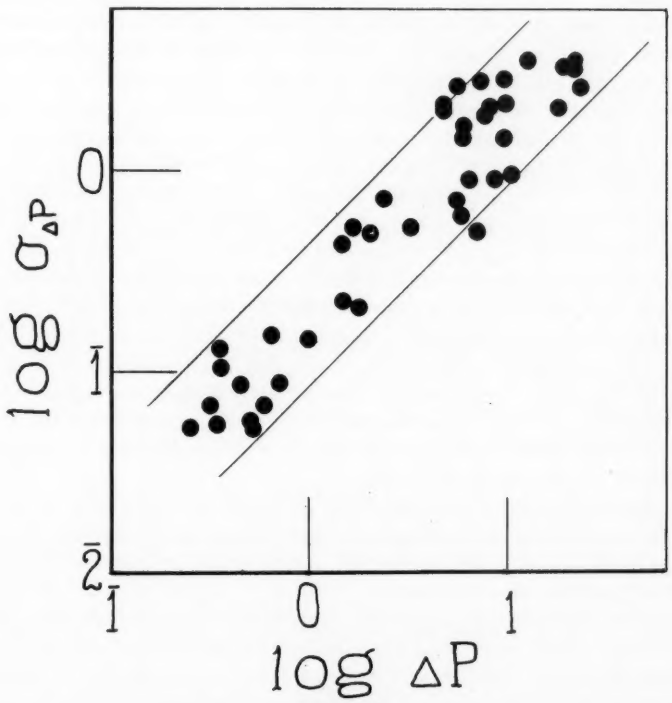

FIGURE 3

Data of the present experiment: relation between mean ΔP (n = 10) and $\sigma_{\Delta P}$ for somaesthetic pressure, P_1 constant and area of application A varied. (The prime on P' signifies that the ΔP corresponds to a *fixed* value of P_1.) The slope of the band = 1.

of 1 gm./sec.^{4} to determine W_2. The method was thus that of continuous change at a "constant" rate in the direction of increase. The initial pressure was P_1 = 6 gm./cm.^{2}, A varying from 12.56 cm.^{2} to 0.41 cm.^{2} in six steps. Six series of determinations were made. The data relative to ΔP and $\sigma_{\Delta P}$ are given (Fig. 3) upon a logarithmic grid. This function has the properties usually exhibited by such data: the band has a statis-

tically constant width, and its slope = 1; ΔP and $\sigma_{\Delta P}$ are directly proportional, and the graph passes through the origin. There is a slight "practice effect" in the scatter of the magnitudes of $\sigma_{\Delta P}$, but this does not bear upon the present argument. The dependence of ΔP upon A, P_1 fixed, is very similar to that obtained in retinal excitation.[10] Over the short range of A open for analysis, log ΔP is an approximately rectilinear declining function of log A with the slope a little greater than 1; there is indication that with still smaller areas the slope may increase, as in vision.

From the slope $d\sigma_{\Delta P}/d(\Delta P)$—the axis intercept of the central line on the log grid (Fig. 2)—a weighted coefficient of variation ($C.V. = 100\ \sigma_1/M$) is readily obtained. This works out to be 20 per cent or a little higher. In Stratton's data[5] it is about 21 per cent (calculated from the relationship between $A.D.$ and σ_1). This is also true when speed of application of ΔW is the experimental variable. In the experiments of Dodge and Gatti[6] the same magnitude of $C.V.$ is apparent. In various experiments with lifted weights[6,7] $C.V.$ = 20 to 21 per cent. Thus in some six series of independent experiments involving quite different technical procedures and different observers, the relative variability of ΔW (i.e., of ΔP) for mechanical (pressure) stimulation of skin and muscle exhibits a definite constancy. The presumption that this constancy may be of general import is emphasized by the fact that in auditory intensive discrimination $C.V.$ for ΔI is of the order of 12 to 15 per cent[1,8] and for visual intensive discrimination is 10 per cent or less,[1] again without regard to details of experimental procedure.

Evidence of this sort, together with the behavior of $\sigma_{\Delta P}$, is to be added to that obtained from a variety of other sources which also consistently show the inadequacy of regarding the variation of intensive discrimination as due to extraneous experimental "errors." There may be mentioned the various cases arising in measurement of visual ΔI, and the calculation of ΔI from variation data of other tests;[9] the behavior of critical flicker frequency and its variability;[12] the relation of σ_{I_c} for flicker to temperature;[12] the properties of σ_θ for the geotropic orientation of rats,[11] and its behavior under experimental treatment; as well as the nature of σ_θ in tests of post-contraction of arm muscles.[13]

Measurements of intensive discrimination by the "method of constant stimuli" show clearly enough that variation in the organism's discriminatory performance does occur; I' and I'', two physically equal standard and comparison stimuli, are not always so judged. It is a different matter, however, to prove that this capacity for variation is intrinsically responsible for the interdependence of ΔI and $\sigma_{\Delta I}$ in tests by other procedures. The properties of ΔP do supply evidence of this sort. It is significant that these indications are consistent with others derived from quite different sources.[1,5,6] The variability of discriminatory performance, an organic

property, is (1) lawful in its manifestations and (2) basically significant for the interpretation and use of data involving sensorial discrimination.

5. *Summary.*—When area of application on the skin (A) is varied and the mechanical pressure P_1 is kept constant by appropriate alteration of weight W_1, ΔP declines with increase of A (and W); ΔP and $\sigma_{\Delta P}$ are nevertheless in direct proportion. The latter relationship cannot be due to "experimental errors," but must be understood as resulting primarily from the organic variability of sensory discrimination. Other evidence is consistent with this view.

[1] Crozier, W. J., *Jour. Gen. Physiol.*, **19,** 503 (1935); Crozier, W. J., and Holway, A. H., *Proc. Nat. Acad. Sci.*, **23,** 23 (1937); Holway, A. H., *Jour. Opt. Soc. Amer.*, **27,** 120 (1937).

[2] Crozier, W. J., *Détérminisme et variabilité*, Paris: Hermann. Pp. 56 (1935).

[3] Muller, G. E., *Zur Grundlegung der Psychophysik*, Berlin: Theo. Hoffmann (2nd printing). Pp. xvi + 424 (1879).

[4] Holway, A. H., and Crozier, W. J., *Psychol. Rec.*, **1,** 170 (1937).

[5] Stratton, G. M., *Philos. Stud.*, **12,** 525 (1896).

[6] Holway, A. H., Harvard University Ph.D. Thesis, Harvard College Library, pp. 65 (1936); Holway, A. H., and Hurvich, L. M., *Jour. Psychol.* **4,** 309 (1937); Gatti, A., and Dodge, R., *Arch. f. d. ges. Psychol.*, **69,** 405 (1929).

[7] Holway, A. H., Smith, J. E., and Zigler, M. J., *Jour. Exper. Psychol.*, **20,** 371 (1937); Holway, A. H., Smith, J. E., and Zigler, M. J., *Ibid.*, (in press) (1937).

[8] Churcher, B. G., King, A. J., and Davies, H., *Phil. Mag.*, **18,** 7th series, 927 (1934); Upton, M., and Holway, A. H., *Proc. Nat. Acad. Sci.*, **23,** 29 (1937); Upton, M., and Holway, A. H., *Ibid.*, **23,** 32 (1937); and further experiments which we shall discuss in another place.

[9] Crozier, W. J., Wolf, E., and Zerrahn-Wolf, G., *Jour. Gen. Physiol.*, **20** (1936–37).

[10] Cf. Holway, A. H., and Crozier, W. J., *Psychol. Rec.*, **1,** 177 (1937); cf. also data in Steinhardt, J., *Jour. Gen. Physiol.*, **20,** 185 (1936).

[11] Crozier, W. J., and Pincus, G., *Jour. Gen. Physiol.*, **6,** 789 (1928).

[12] Crozier, W. J., Wolf, E., and Zerrahn-Wolf, G., *Jour. Gen. Physiol.*, **20,** 393, 411 (1937); and *Ibid.*, **21** (1937, in press).

[13] Holway, A. H., Crolius, M. F., Pratt, J. E., and Zigler, M. J., *Jour. Psychol.*, **3,** 491 (1937).

SPECIFIC CONSTANTS FOR VISUAL EXCITATION

By W. J. Crozier, Ernst Wolf and Gertrud Zerrahn-Wolf

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Communicated August 4, 1937

I. Frequency of interruption and critical intensity of flashing light determining threshold response to intermittent illumination are interrelated in a definite manner which has been established for several kinds of animals. This has been possible by utilizing reactions to a moved set of stripes alternately transparent and opaque.[1] For a number of reasons, partly having to do with predictions by certain theories of the marginal recognition of flicker, it was desirable to explore the dependence of mean critical illumination I_m upon flicker frequency F under various conditions for the case in which the durations of light (t_L) and of darkness (t_D) in a flicker cycle are equal. Experiments in which the ratio t_L/t_D is made to differ from 1 give important evidence concerning the physical basis of response to flicker.[2] The properties of the F/I_m curve as related to temperature[3] are of equal and similar theoretical import. An interpretation of the F–I function has been proposed[4] in terms of the ascertained properties of intensive discrimination, discrimination of flicker being regarded as a limiting case for discrimination of intensities.[5] The establishment of a degree of excitation leading to marginal recognition of flicker is taken to involve the summation of effects due to a logarithmic frequency distribution of excitable elements. This is consistent with the fact that a probability integral adequately formulates the connection between $\log I_m$ and F,[6] when the situation is not complicated by structural factors (as revealed by separate experiments[7] in the case of arthropod eyes).

These considerations require an essentially statistical[8] conception of the summated elements of excitation, rather than an anatomical theory of end organ units. Excitation, with reference to response, is necessarily defined in terms of units based upon the relation between excitation and reaction. Since in addition the basic quantitative properties of acts of intensive discrimination are clearly the same for diverse modes of sensory excitation[8] and in diverse organisms, the chance of obtaining from intensive discrimination immediately pertinent evidence as to the physico-chemical mechanism of excitation in any given case would seem to be quite remote.[9]

It is important for the statistical formulations of behavior that the descriptive constants they involve and their properties can be shown to derive from specific properties of individual organic systems.[10] To the extent that such demonstrations are possible, the parameters concerned must be accorded the status of organic invariants.[11] The characterization of such invariants becomes a prime objective of physiological inquiry. The demon-

stration of this kind of invariance can be provided in a visual flicker experiment (1) if animals of a given specific type give homogeneous data[12] exhibiting a consistent mode of dependence of I_m upon F, and (2) if the constants in the formulation of this relationship exhibit unitary behavior in inheritance.[13] When an application of test (2) does not give a simple answer, nothing is established one way or the other—the matter may be so complex as to require more elaborate analysis; where it does give a clean-cut answer, the probability is great that the constants involved are determined by

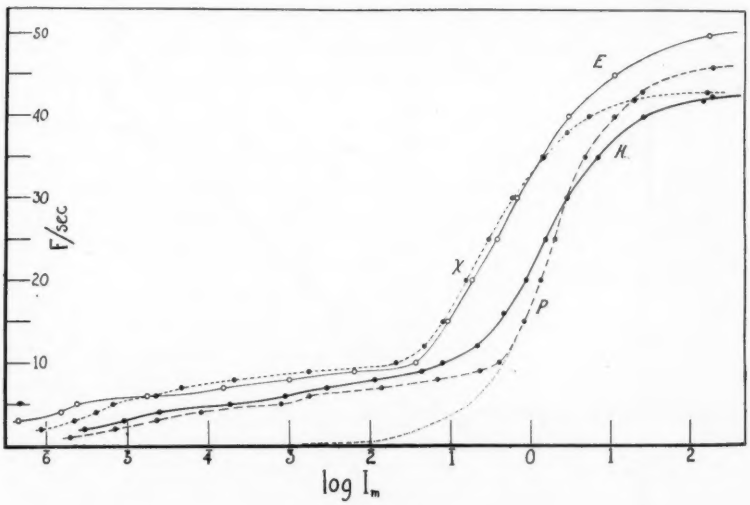

FIGURE 1

Log I_m for four types of teleosts: E = *Enneacanthus* (Sunfish); X = *Xyphophorus helleri*; P = *Platypocoelis maculatus*; H = extracted backcross hybrid from X and P. The downward extrapolation of the probability integral describing the "cone" part of the curve in each case encounters the axis in the place required to account for the hump on the flat portion of the graph; this is shown for P.

Each plotted point is the average of one or more sets of 30 to 36 homogeneous readings each of critical illuminations on 10 or 12 individuals, with 50 per cent light time in a flicker cycle, at 21.5°.

the specific organization of the reacting system concerned rather than, for example, by statistical accidents of the observational procedure. This test has been made in a preliminary way[14] with the F–I_m curves for certain fresh water teleosts, which permit interspecific cross-breeding. The result may be taken to illustrate a method of investigation which has large possibilities.

II. With the procedure described elsewhere,[15] mean critical illuminations (I_m) for response to flicker were measured as a function of flicker fre-

quency (F) for a number of teleost species. The curves (Fig. 1) are characteristic, and are to a high degree reproducible. The variability of the individual measurements is discussed in our more complete reports.[14]

The curve labeled H is based upon the performance of individuals resulting from the backcross $F_1 \times Xiphophorus$, where $F_1 = Xiphophorus$ (X) $\times$ *Platypocoelis* (P). Curve H rises to a maximum which is that of X;

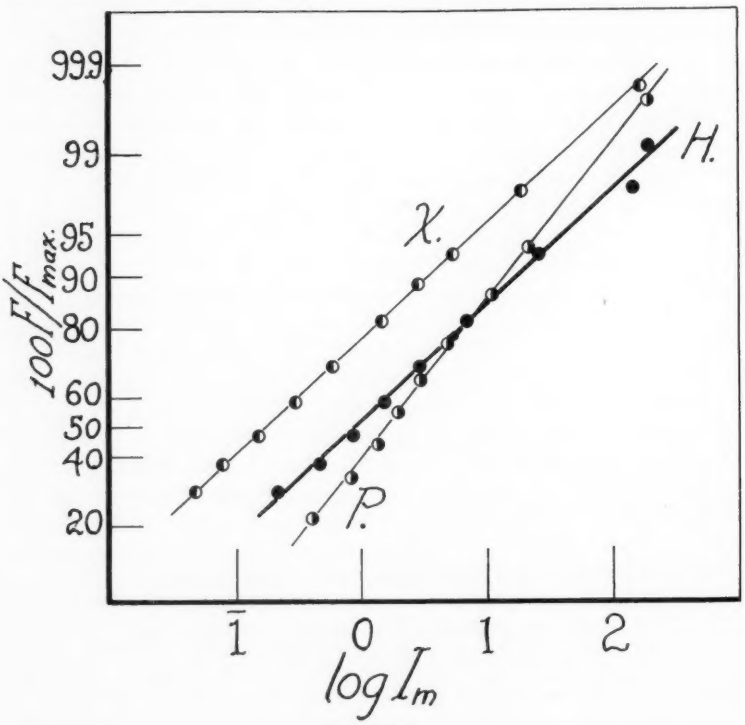

FIGURE 2

The flicker-response data, cone portion, for X, P and H on a probability grid. The slope (i.e., $\sigma_{\log I}$) is the same for X and for H. F_{max} is $X = 43.1$; $H = 43$; $P = 46.1$.

the maximum for the "rod" portion of the curve, however, is that for the P stock, and the general position on the intensity axis is more like that of P than of X.

The composite curves of I_m vs. F can each be separated (as shown in Fig. 1) into two portions on the basis that the upper ("cone") part is fitted by a log probability integral; the downward extension of the curve approaches the log I axis in precisely the region required to account for the

"hump" in the flattish part of the composite graph. The "rod" portions of the curves are also well described by a curve of this kind, as likewise the descending limbs of the difference curves which presumably indicate the manner in which increasing illumination brings about the dropping out of rod contributions to the determination of the critical intensity.

The point we desire to illustrate is contained in figures 2 and 3. The observations have been plotted upon a probability grid in which 100 F/F_{max} is the ordinate and log I_m is the abscissa. The slope of the line is an inverse measure of the breadth ($\sigma_{log\ I}$) of the assumed underlying frequency distribution of elemental contributions to the determination of the effect eventuating in the discriminatory response. The value of log I_m for which 100 F/F_{max} = 50 is a measure of relative excitability (a kind of chronaxie, τ'). The value of F_{max} and the value of $\sigma_{log\ I}$ for the cone portions of the curve are identical in H and in X despite the intermediate value of τ'. For the rod portions, $\sigma_{log\ I}$ is also the same in H and X, although F_{max} is identical to that in P and τ' is again intermediate. (The curves for declining rod participation with higher intensities also show this kind of correspondence.)[14] The values of these constants for E are quite different.

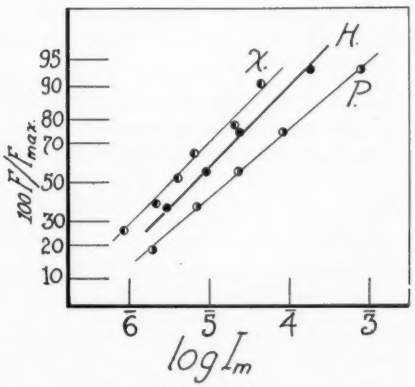

FIGURE 3

The flicker-response data, rod portion, for X, P and H. (The departures are of course magnified by the percentage scale.) The slope is again the same for X and for H. F_{max} is X = 7.7; H = 5.36; P = 5.36.

Such facts speak for the independent hereditary determination of properties respectively associated with rods and with cones, and for the independence of σ, τ' and F_{max}.

III. Since the F–I_m curves are not the same for various teleost types, the occurrence of quantitative agreements in the values of descriptive parameters among generations involved in breeding experiments must be due to inherited parallelisms in organic constitution. The present experiment indicates that these constitutional invariants may be subject to comparatively simple rules of inheritance, and thus that specific constants derived from a probability conception of the basis for behavioral performance may be organically determined. They thus measure essential features of organization. This is supported by the fixity of such parameters in a given organism under conditions of altered temperature and[16] altered values of the

proportion of light time to dark time in a flicker cycle,[17] which produce different kinds of changes in the F–I_m curve. A factual basis is thus provided for theoretical objection to the not uncommon practice of averaging data from different individuals not known to be genetically alike with reference to the property being measured. The reciprocal interest of such considerations for genetical experiments is sufficiently clear. The work is being continued.

[1] Wolf, E., *Jour. Gen. Physiol.*, **17,** 7 (1933–34); Wolf, E., and Crozier, W. J., *Ibid.*, **16,** 787 (1932–33); Wolf, E., and Zerrahn-Wolf, G., *Ibid.*, **19,** 495 (1935–36); Crozier, W. J., Wolf, E., and Zerrahn-Wolf, G., *Ibid.*, **20,** 211, 363 (1936–37).

[2] Crozier, W. J., Wolf. E., and Zerrahn-Wolf, G., *Ibid.*, to be published (1937–38).

[3] Crozier, W. J., Wolf, E., and Zerrahn-Wolf, G., *Ibid.*, **20,** 393, 411 (1936–37).

[4] Crozier, W. J., *Ibid.*, **19,** 503 (1936–37); *Proc. Nat. Acad. Sci.*, **22,** 412 (1936); *Ibid.*, **23,** 71 (1937); and footnote 3.

[5] Cf. footnote 4.

[6] Crozier, W. J., *Proc. Nat. Acad. Sci.*, **23,** 71 (1937); Crozier, W. J., Wolf, E., and Zerrahn-Wolf, G., *Jour. Gen. Physiol.*, **20,** 363, 393, 411 (1936–37). (We have also used, solely for convenience, a logistic grid.)

[7] In course of publication, *Jour. Gen. Physiol.*

[8] Crozier, W. J., *Proc. Nat. Acad. Sci.*, **22,** 412 (1936); *Jour. Gen. Physiol.*, **19,** 503 (1936–37); Crozier, W. J., and Holway, A. H., *Proc. Nat. Acad. Sci.*, **23,** 23 (1937).

[9] Crozier, W. J., and Holway, A. H., *Ibid.*, **23,** 509 (1937); Upton, M., and Crozier, W. J., *Ibid.*, **22,** 417 (1936).

[10] Crozier, W. J., and Pincus, G., *Jour. Gen. Physiol.*, **13,** 57, 81 (1929–30); *Ibid.*, **15,** 421 (1931–32); *Ibid.*, **20,** 111 (1936–37).

[11] Crozier, W. J., in *Handbook Exptl. Psychol.*, Ed. Murchison, Clark University Press (Worcester) (1929); *Détérminisme et Variabilité*, Paris, Hermann, 56 pp. (1935).

[12] Crozier, W. J., *Proc. Nat. Acad. Sci.*, **22,** 412 (1936).

[13] For analogous cases in other phenomena, cf. footnote 10 and Pincus, G., *Jour. Gen. Physiol.*, **14,** 421 (1930–31).

[14] Data in press in *Jour. Gen. Physiol.*

[15] Crozier, W. J., Wolf, E., and Zerrahn-Wolf, G., *Jour. Gen. Physiol.*, **20,** 211, 411 (1936–37).

[16] *Ibid.*, **20,** 393, 411.

[17] To be published.

THE RATES OF ABSORPTION OF RADIO-SODIUM IN NORMAL HUMAN SUBJECTS*

BY JOSEPH G. HAMILTON

Communicated June 7, 1937

The internal admistration of radio-sodium in the form of sodium chloride was first performed at the University of California Hospital in March of 1936 upon a subject with lymphatic leukemia. The radioactivity of the patient and his excreta was observed in addition to any possible clinical responses. A second patient with a myelogenous leukemia received the same substance, but of considerably greater activity. No definite clinical improvement or toxic effects were observed; but data referable to the concentration of the radio-sodium in the body fluids and its excretion were obtained from these studies and are reported elsewhere.[1]

Following the intraduodenal administration of the radio-sodium to the second patient it was found that for the succeeding 8 hours the measured activity of his body was greater than was to be expected, allowing for the gamma radiation absorbed by the tissues and the amount of radio-sodium lost by excretion. Recently a third patient, suffering from a reticulum-cell sarcoma of the cervical lymph nodes, was given the activated salt by mouth and the same phenomena were observed for a period of 13 hours.[2] This apparently anomalous behavior of the radio-sodium when given by mouth or by duodenal tube, as contrasted to the results obtained after intravenous administration, was thought to be due to the fact that it was more slowly absorbed than might be expected. This problem has been investigated further by studies concerning the apparent rates of absorption of radio-sodium in normal human subjects.

Preparation of the Radio-Sodium.—The radio-sodium was prepared by the deuteron bombardment of crystalline sodium caloride using the magnetic resonance accelerator which was developed by Lawrence and Livingston[3] and later re-designed by Lawrence and Cooksey[4] of the Radiation Laboratory of the University of California at Berkeley. This apparatus, commonly described as the "Cyclotron," has been constructed for the multiple acceleration of light ions to high speeds. The deuterons spiral around within a drum-shaped chamber lying between the poles of a powerful electromagnet. The combined action of the magnetic field and a high frequency oscillating electric field upon these particles produces progressive increments of velocity with every revolution of the ions within the chamber so that their terminal energy upon emerging at the periphery is in the neighborhood of 6,000,000 electron volts. These rapidly moving particles, which consist of a proton and a neutron bound together and are isotopes of ordinary hydrogen ions, stream out of the cyclotron as a narrow beam which is directed

against the substance to be activated. When one of these particles approaches the nucleus of a sodium atom the proton is repelled by the intensely positive field and the neutron is captured by the nucleus. The sodium then becomes heavier by the weight of the neutron and its atomic weight increases by one without altering its chemical properties. This isotope of

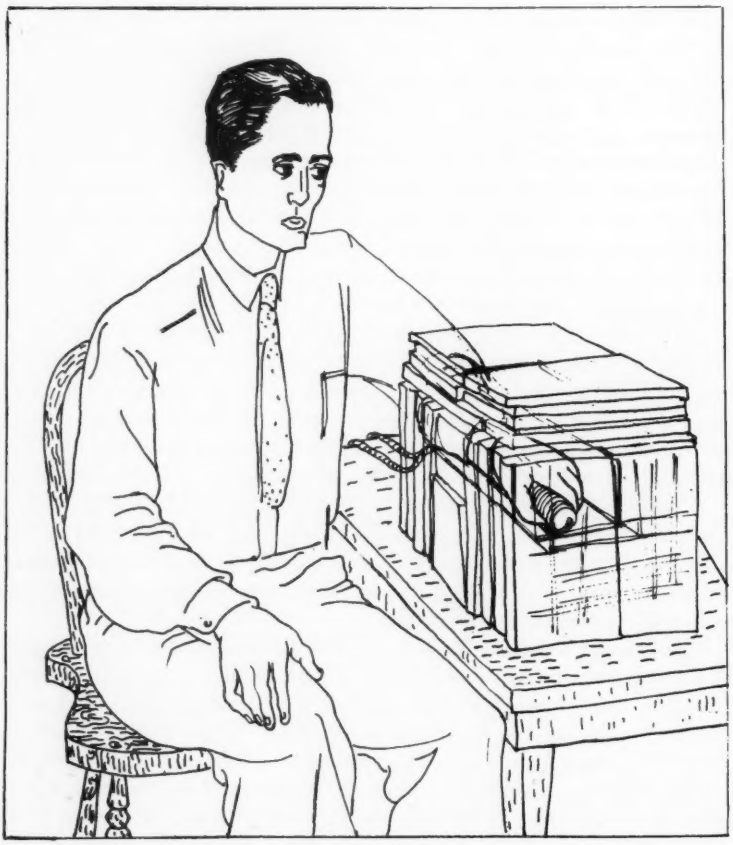

FIGURE 1

sodium does not occur in nature and is unstable; in the course of 14.8 hours, half of its atoms emit an energetic beta ray. When the sodium nucleus loses the electron (i.e., beta ray), magnesium is formed which immediately releases its excess of energy as a gamma ray. These gamma rays enable the investigator to measure quantitatively the amount of radio-sodium in a given sample. The interval of time between the formation of the active

magnesium and the emission of its gamma ray is so brief as to be of no consequence, and therefore the distribution of radio-sodium in any biological system can be considered as a measure of the metabolism of the naturally occurring sodium ion. The only possible exception to this view is that the radiations from the activated sodium might alter the function of the organism, or a portion of its cellular components. Colwell,[5] in his excellent monograph, discusses the effect of radiations upon cellular structures and their metabolism. However, the degree of exposure with both beta and gamma rays necessary to accomplish measurable changes was very much greater than was used in the treatment of the previously described patients with radio-sodium. The quantity of the activated salt given to the majority of the subjects in the absorption experiments was from $^1/_{600}$th to $^1/_{1500}$th the amount given to the third patient.

Method of Study and Administration.—In the following experiments eight normal human subjects were used, two of them being women. The radioactivity of the hand, after the subject had ingested the radio-sodium by mouth, was used as the "indicator" of absorption. A small hollow lead box with walls of 6 inches in thickness, open at one end and with sufficient space inside to admit the subject's hand and forearm, was used (Fig. 1). This arrangement permits the accurate measurement of very minute quantities of any radioactive substance in the blood and at the same time shields the hand and measuring instrument from the activity of the remainder of the body. A Geiger-Müller counter with a constant voltage supply was used in all of these experiments. The counting chamber was 1 by 4 inches in size and was held in the hand within the lead box. The activity was recorded as impulses per second in the counter circuit.

The radio-sodium was prepared by the deuteron bombardment of from 1 to 2 gm. of crystalline sodium chloride. The sample was then allowed to "age" for at least 90 minutes to permit the decay of the radio-chlorine and then its activity was determined with a quartz fibre electroscope which had been previously calibrated against a 1 mg. mesothorium standard. The salt was then dissolved in a measured amount of water, usually 50 or 100 cc., and then a sufficient amount of the solution measured out so as to give each subject the necessary quantity of radio-sodium to be taken. At the start of each experiment the subject took his station with one hand inside the lead box and the sample to be swallowed in the other, and several background readings were taken. Then the solution was ingested and followed by a glass of water to wash all traces of the radio-sodium out of the mouth and esophagus. Readings from the counter were taken immediately afterward and continued every few minutes for the next hour. A second subject took his place and then drank the activated solution and at the end of the next hour exchanged places with the first individual. In this manner it was possible to carry out these studies upon two subjects

at a time. The first few experiments were conducted for a period of from 6 to 7 hours; but since absorption in some instances did not appear to be completed at the end of this time, the later studies were extended to 10 hours.

The first subject received 2000 μc.e.† but it was subsequently found that from 80 μc.e. to 200 μc.e. would give just as satisfactory results. Whenever possible the subjects were in the fasting state for at least 4 hours before taking the radio-sodium. However, it was observed that the taking of food an hour or so before the experiment made no appreciable difference. During the course of the experiments a short period was allowed for the subjects to partake of a light meal and, in this instance as well, no alteration

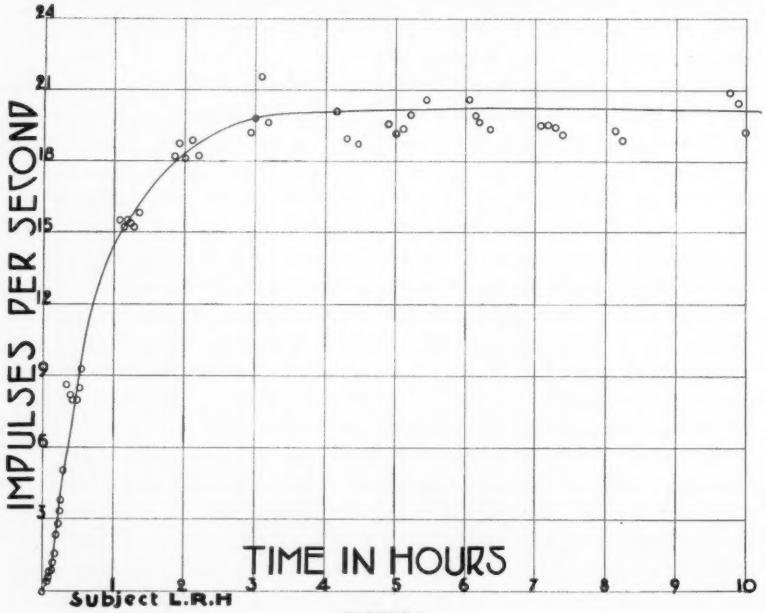

FIGURE 2

in the absorption curves was to be noted. The studies were on the top floor of a large building, and when a subject walked rapidly up the four long flights of stairs from the ground floor a definite but brief increase in the activity of his hand was observed.

Six subjects received the radio-sodium but once and the remaining two took the salt twice. The absorption curves of the individuals receiving the radio-sodium a second time were no more similar upon the second study than the curves of the different subjects.

Two typical absorption curves are shown in figures 2 and 3; the abscissa represents the intervals of time following the ingestion of the radio-sodium

and the ordinate the impulses of the counter circuit per second after correction for the decay of the radio-sodium has been made. The interval of time following the taking of the radio-sodium, but before any definite activity could be detected in the hand, varied from $2^1/_2$ to 9 minutes. In figure 2 the initial portion of the curve is quite steep but at the end of 3 hours it becomes flat, suggesting that absorption was completed at the end of this period of time. The beginning of the second curve shown in figure 3 is less sharply inclined, but at the end of 10 hours it has not become level. This gradual increase of activity at the termination of the experiment has

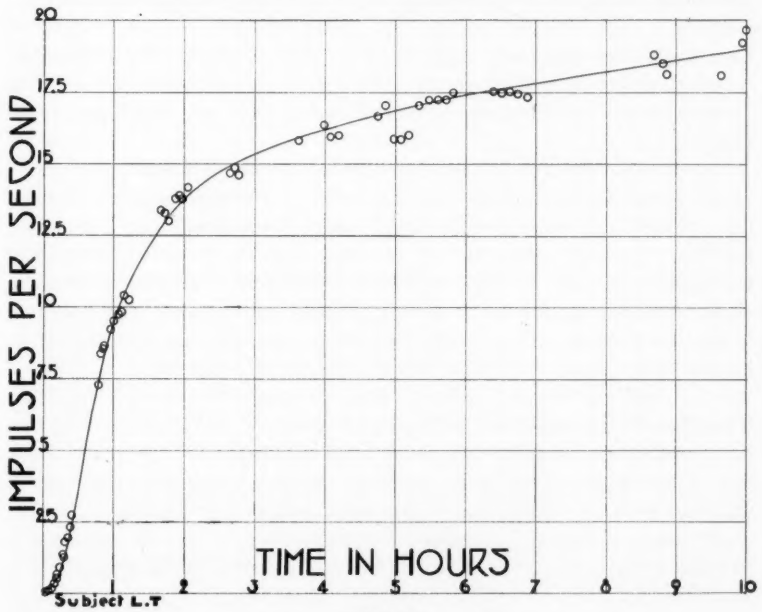

FIGURE 3

been observed in several other subjects but does not appear to be related to the slope of the initial part of the absorption curve. In future studies the period of observation is to be prolonged until absorption has been completed in all the subjects studied.

Discussion.—Before any conclusions can be drawn, several factors must be considered. The results are merely the successive measurements of the radioactivity of the subjects' hands. In other words, the hand is an "indicator" of absorption, but not necessarily a quantitative index of the rate that the radio-sodium passes from the digestive tract into the blood stream. The hand is a complex structure composed of many histological entities

and into these tissues the atoms of radio-sodium diffuse from the blood. If the rate of diffusion is rapid in the hand then the counter will, in effect, measure the rate of change in activity of the incoming blood stream. However, if the rate of diffusion is slow, in comparison to the rate of absorption, the initial portion of the curve will be flatter and the interval of time before the maximum rise occurs will be prolonged. Alteration of the caliber of the blood vessels will change the total volume of blood in the hand and thereby produce variations in the measurements. An example of this factor was observed following exertion in one of the subjects, and has been previously described. To avoid this source of error the subjects were not exposed to any undue temperature changes and the activity determinations were made only when they were at rest. The possible influence from the accumulation of sweat from the subjects' hands upon the counter tube was determined at the conclusion of several experiments and was found to be negligible.

The rate of diffusion of the radio-sodium into the tissues and their fluids of the remainder of the body may also affect the apparent rate of absorption. If diffusion is rapid this variable can be overlooked; but if it should be slower, yet faster than the rate of absorption, the observed curves will rise more gradually to a maximum. Should the rate of diffusion be slower than absorption then the curve would soon decline after reaching a maximum as the radio-sodium in the blood stream was being taken up by the tissue fluids. Another possible variable is the state of sodium "excess" and "deficit" of the subject at the time when the activated salt is taken. It is reasonable to suppose that the rate of absorption would be more rapid in an individual in a state of sodium "deficit." The influence of loss of the radio-sodium from the body through the various channels of excretion is of no importance during the length of time that these experiments were conducted. The rate of excretion of radio-sodium when given to patients has been found to be less than 0.2 per cent per hour and so the amount lost at the end of 10 hours would be less than the experimental limits of error.

A consideration of the possible factors which might produce significant alterations in the observed rates of absorption leads to the conclusion that the most important variables are the quantity of sodium in the body at the time of experimental study and the rate of diffusion of sodium from the blood stream into the other body structures. Very little is known about the first factor; but it is generally assumed that practically all of the body's sodium is contained in the body fluids, which comprise about 20 per cent of the total body weight, and that diffusion is rapid. Even less information is available as to the effect of the total quantity of sodium in the body upon the rate of absorption. The latter factor can be eliminated as a source of error by placing the subjects upon a fixed sodium intake for a

period of time, and after they are in a state of sodium "balance" (i.e., sodium intake equals sodium excretion) for several days, determining the rates of absorption. At present it is not possible to determine the rates of diffusion in normal human subjects; but future studies are planned in conjunction with further clinical investigations upon the metabolism of sodium following administration of radio-sodium to leukemic patients at the University Hospital in the attempt to obtain more precise information upon this question.

Summary.—1. The rate of absorption of sodium has been studied following the oral administration of its radioactive isotope to normal human subjects.

2. Absorption of the radio-sodium has been observed to begin within a few minutes and is apparently completed in a period of from 3 to 10 hours in some subjects, but in others equilibrium was not reached at the end of 10 hours.

3. A new method of study of the metabolism of sodium has been described. This technique may be adapted to other artificially radioactive elements, or their compounds, when given by mouth or by other channels of administration.

Acknowledgment.—The author wishes to express his appreciation to Dr. Robert S. Stone of the Department of Roentgenology for his encouragement and advice, and to the individuals whose willing coöperation as experimental subjects made this work possible. The radio-sodium was supplied through the courtesy of Prof. Ernest O. Lawrence and his coworkers of the Radiation Laboratory of the University of California and through the support of the Josiah Macy, Jr., Foundation.

* From the Division of Medicine, University of California Medical School, San Francisco, California; and from the Radiation Laboratory, University of California, Berkeley.

† Microcurie equivalents.

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THE DEPOSITION OF RADIO PHOSPHORUS IN TISSUES OF GROWING CHICKS

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Introduction.—The artificially induced radioactive elements behave chemically as their non-active relatives, and after feeding, they can be detected and quantitated in the various tissues by means of an electroscope or Geiger counter. This introduces a new method for the study of the metabolism of many of the elements, since these radioactive isotopes are "tagged" and can be traced. One of these elements of interest to biology is radio phosphorus ($_{15}P^{32}$). Radio phosphorus decomposes into beta rays and sulfur and its half life is 14.5 days. The subsequent fate of radio phosphorus with respect to its deposition, transfers from one tissue to another, biological synthesis of phosphorus containing compounds, and excretion in feces and urine gives information regarding the metabolism of phosphorus which is of value to the biologist.

Chievitz and Hevesy (1935, 1937) have studied the phosphorus metabolism in white rats by feeding radioactive sodium phosphate. Twenty-two days after its ingestion 58.1 per cent was found to have been excreted in the feces and urine. Radio phosphorus was found in all of the tissues examined, a large part of it in the skeleton and muscles. However, on the basis of activity per unit weight, brain, spleen and kidney and liver were found to contain the highest amounts.

Artom, *et al.* (1937), investigated the distribution of radio phosphorus in a rat five days after administration, with particular attention to lipidic and non-lipidic phosphorus. Lipidic radio phosphorus compounds were found in liver, intestine, kidney, parenchyma, muscle and brain and medulla, showing that a synthesis of phospholipids from inorganic radio phosphorus occurs in the body.

In the present investigation, the number of tissues and organs examined has been expanded to a total of thirteen. With the greater activities obtained by the use of the cyclotron (one hundred to one thousand times greater than those available to previous workers), it was possible to investigate tissues containing relatively small amounts of phosphorus and, in addition, to determine with equal convenience the total activities of the tissues four and sixty days after dosage. Thus it was possible to investigate the exchange of phosphorus from one tissue to another with the passage of time. For the purposes of this investigation chickens were used.

Experimental Methods.—The radio phosphorus was obtained from the Radiation Laboratory, University of California. It was fed to growing

TABLE 1

DEPOSITION OF RADIO PHOSPHORUS IN TISSUES*

TISSUE	PER CENT OF TOTAL ACTIVITY IN BIRDS			PER GRAM TISSUE (IN MICROCURIES)—WET WEIGHT			PER GRAM PHOSPHORUS (IN MICROCURIES)	
	4 DAYS	60 DAYS, BIRD 4	60 DAYS, BIRD 5	4 DAYS	60 DAYS, BIRD 4	60 DAYS, BIRD 5	4 DAYS	60 DAYS, BIRD 4
Spleen	0.19	0.034	0.08	0.0088	0.0018	0.0015	3.1	0.6
Bone marrow[1]	0.067	0.044	0.10	0.0017	0.0018	0.001	0.9	1.0
Brain	0.127	0.05	0.14	0.0018	0.0014	0.0014	1.0	0.8
Skeletal muscle and fat[2]	60.0	13.0	26.0	0.0071	0.001	0.001	3.3	0.5
Liver	2.0	0.4	0.87	0.0068	0.0009	0.0007	2.3	0.3
Kidney	0.4	0.08	0.12	0.0047	0.0009	0.0005	2.0	0.4
Heart	0.24	0.1	0.16	0.0027	0.0008	0.0005	1.5	0.5
Testes		0.2	0.34		0.0007	0.0007		0.4
Lung	0.51	0.05	0.14	0.0057	0.0006	0.0006	0.3	0.3
Intestine	3.5	0.17	0.57	0.0028	0.0004	0.0004	1.0	0.2
Gizzard		0.11	0.22		0.0003	0.0002		0.2
Blood[3]	0.97	1.2	0.7	0.0008	0.0006	0.0002	0.8	0.2
Bone	32.0	84.0	70.0	0.17	0.08	0.055	1.0	0.5

* Since the bird analyzed at four days contained a different amount of radio phosphorus and was of a much different body weight than birds Nos. 4 and 5 at sixty days, a comparison between the activities of the various tissues of different birds per gram of tissue or per gram of phosphorus is not feasible. These data are given in this manner so that the activities of the tissues of the *same* bird can be compared. A comparison of the activities of the tissues of different birds can be made when the activities are presented as per cent of the total.

[1] Bone marrow was taken only from tibia and femur of both legs.

[2] Muscle is calculated after weight of other organs, tissues and skeleton is known.

[3] Blood volume is taken as 10 per cent of body weight. Total activity in microcuries of the birds analyzed was: at 4 days, 3.7; at 60 days (4), 8.8; at 60 days (5), 3.

TABLE 2

ACTIVITY OF BONE AND SKELETAL WEIGHT 60 DAYS AFTER LAST FEEDING OF RADIO PHOSPHORUS

BIRD NO.	SEX	ACTIVITY PER GRAM BONE *Microcuries*	WEIGHT OF SKELETON *Grams*	PER CENT OF TOTAL WEIGHT OF BIRD*	TOTAL ACTIVITY OF SKELETON *Microcuries*
1	♀	0.065	32.7	2.55	2.08
2	♀	0.067	54	3.8	3.62
3	♀	0.061	61	5	3.72
4	♂	0.08	93	5.8	7.44
5	♂	0.055	41	3.5	2.1

(All birds received similar amounts of radio phosphorus)

* It will be noted that the skeletal weights of the birds as per cent of total body weight were variable. This is attributed to normal variations which are consistently found in animals of this type. These birds appeared to be normal and enjoyed normal growth. A complete description of the animals, including blood pictures, was given by Cook and Scott (1937).

chicks according to the method outlined in a previous paper (Scott and Cook, 1937). At appropriate intervals the chicks were killed and all of the organs and samples of other tissues removed. The fresh weights were determined immediately, and the tissues ashed. The skeleton was freed from muscle and connective tissue by treatment with aqueous sodium hydroxide. After the bone was cleaned in the above manner, it was washed with a large quantity of water, dried and ground to a fine powder so that an aliquot of the entire skeleton could be obtained for beta-activity analysis. Known amounts of bone and the ash of the various tissues were placed upon glass slides and the activity determined by means of a Lauritzen-type electroscope. With extreme care, activities can be measured correctly to 10^{-4} microcuries. From the standpoint of internal consistency the electroscope values were correct to within three or five per cent. Such an error is not due to any defect or lack of sensitivity on the part of the electroscope, but rather to absorption of beta rays by the ash samples themselves. The upper limit of the energy of P^{32} is about 1.7 million electron volts. However, even particles with such a relatively high energy are stopped by layers of sample. Great care was therefore taken in preparation of samples to keep the weight as small as possible and of the same thickness.

Results and Discussion.—In table 1 are presented data showing location of radio phosphorus in various tissues, in one chick four days and in two chicks sixty days subsequent to the administration of the radio phosphorus. The results are expressed in three ways: (1) as per cent of total activity found in various organs and tissues, (2) as activity per gram of tissue and (3) as activity per gram of total phosphorus from various tissues. The following points in connection with these data warrant consideration. Further data are given in connection with particular points as they arise.

1. The absorption of radio phosphorus was determined in all cases by subtracting the activity of that radio phosphorus which was excreted or uneaten from the known original activity of the sample prior to administration. This method was checked upon the bird which was killed 4 days after radio phosphorus administration. Ninety-two per cent of the total radio activity was accounted for. Table 3 shows an average absorption of radio phosphorus of 77.2 per cent. The unabsorbed balance was lost partially in that portion of the feed which was not eaten and which contained radio phosphorus, and partially in the feces and urine.

Since ordinary red phosphorus ($_{15}P^{31}$) was used to produce the radioactive isotope ($_{15}P^{32}$) each sample contained inactive phosphorus in much greater quantity than radio phosphorus. Therefore, because the phosphorus storage in an animal is limited, the per cent of retention of the radio phosphorus will be inversely proportional to the amount of inactive phosphorus fed with it.

2. Radio phosphorus was distributed throughout all of the tissues ex-

amined four days and sixty days after ingestion. However, the distribution of relative amounts of radio phosphorus varies considerably and the passage of time allows these differences to become very distinct.

3. At four or sixty days after radio phosphorus feeding, the major part of the radio phosphorus was found in the bones and in muscle. These results need little explanation because of the high phosphorus content of bone, and the great weight of muscular tissue relative to the weight of the various organs in the body.

4. More than four days were necessary for the radio phosphorus to reach a state of quasi-equilibrium in most of the tissues, although the concentration of radio phosphorus in blood was quite constant from the standpoint of per cent of the total activity present in the bird.

5. In per cent of total the greatest shift of radio phosphorus occurred in the digestive tract. The relatively high per cent in the beginning was probably due to the recent absorption of the radio phosphorus through the gut

TABLE 3

ABSORPTION AND EXCRETION OF RADIO PHOSPHORUS

AGE OF BIRDS WHEN GIVEN	ACTIVITY OF SAMPLE	PER CENT RETAINED	PER CENT EXCRETED
Days	*Microcuries*		
11	35	86	14
29	260	77	23
50	180	70	30
72	190	70	30
78	960	83	17
Average		77.2	22.8

wall and to the fact that it was there least diluted with the phosphates already present in the body. Between these two sources of phosphorus the tissues can show no discrimination.

6. All the other tissues with the exception of bone showed a relative decrease in per cent of radio phosphorus and of these muscle is the most striking example. At four days this tissue contained sixty per cent of the total and at sixty days only thirteen and twenty-six per cent (in two birds, respectively).

7. When comparison was made of the activity per gram of tissue, bone was highest at four days and blood was least. Sixty days after administration bone was even relatively higher, and blood, gizzard and intestine were least. Furthermore, brain and bone marrow changed from relatively low to relatively high activities. This result may be referable to the high phospholipid content of brain and bone marrow, although such a suggestion should be checked by analyses of the phosphorus compounds

in these tissues. The idea receives further support, however, from the distribution of the radio phosphorus per gram of various tissues sixty days subsequent to its ingestion, for there appears to be a rough correlation between these values and the phospholipid content of the tissues (brain and bone marrow are definitely greatest).

8. Although the amount of radio phosphorus in bone was high initially (32 per cent of the total radio phosphorus retained by the bird), this value increased greatly after 60 days to 84 per cent and 70 per cent for two birds, respectively. The high activity values of bone shown in table 1 were confirmed by analyses of three other birds, all of which received similar amounts of radio phosphorus. The data secured on all five birds are given in table 2. It will be seen that the activity per gram of bone is quite constant, regardless of the weight of the skeleton. It must be remembered, however, that the total skeletal weights given in table 2 are the values obtained after the bone had been treated with aqueous sodium hydroxide. Such treatment removes the major part of the organic material which constitutes approximately 27 per cent of the total weight of bone. It seemed advisable, however, to consider as bone the residue, mainly tricalcium phosphate, which remained after alkaline extraction. The large amount of radio phosphorus in the tissue may be accounted for by the exceptionally high phosphorus content of bone. This is in conformity with the finding that the ratio of radio phosphorus per gram phosphorus is not materially higher in bone than in other tissues.

The work reported here is in agreement with that of Chievitz and Hevesy, and Artom, *et al.*, in so far as comparisons are possible. Although we were able to show better storage of radio phosphorus in the skeleton, this may be explained by the fact that the chicks used by us were growing rapidly and depositing large amounts of phosphorus in the skeleton.

Summary.—1. A study of the retention and deposition of radio phosphorus was made upon growing white leghorn chicks.

2. The activities of the various tissues were due to beta radiation from radio phosphorus ($_{15}P^{32}$) and are expressed in terms of microcuries.

3. Radio phosphorus was deposited in all tissues examined, but principally in the skeleton and the musculature.

4. The activites of the thirteen tissues examined were of the same order of magnitude with the exception of bone when compared upon the basis of activity per gram wet weight.

5. When the phosphorus content of the various tissues, including bone, is taken into consideration, the activities of units of phosphorus, regardless of their source, are quite similar.

6. A comparison of the relative activities of radio phosphorus in tissues at 4 and 60 days showed marked shifts of radio phosphorus principally from

muscle and intestine to bone, bone marrow and grain. Spleen showed a high radio phosphorus content at all times.

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